BIOLOGY OF AFRICANIZED AND EUROPEAN HONEY BEES, *Apis mellifera*, IN VENEZUELA

By

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"He must be a dull man who can examine the
exquisite structure of a comb, so beautifully
adapted to its end, without enthusiastic admiration."

Charles Darwin 1859

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To determine factors responsible for the greater success of Africanized honey bees, Apis mellifera, in tropical regions of South America, demographic parameters affecting colony reproductive rates were evaluated for Africanized and European honey bees under identical conditions in Venezuela. Worker bee development time was evaluated as an interaction between egg genotype, comb cell size and nurse bee genotype. Africanized worker bees developed faster than European bees: 18.9 and 19.8 days, respectively. There was no significant effect of comb cell size or nurse bee genotype. Mortality for different developmental stages was recorded. The relationship of worker bee development time to colony growth rate is discussed.

Africanized queens develop in 14.5 days post-oviposition compared with 15.0 days for European queens. Queen pupal weights were not significantly different. Post-emergence maturation rates for Africanized and European queens were similiar as determined by both the

ages when queens attracted drones and the ages when oviposition was initiated.

Daily egg laying rates and brood production during initial colony growth were not significantly different for Africanized and European queens. Africanized and European worker bees did not differentially affect egg laying and brood production rates.

Differences in reproductive rates between Africanized and European honey bees in South America cannot be attributed to differences in intrinsic demographic factors. A hypothesis based on differences in resource utilization efficiency is presented to explain the success of Africanized bees compared with European bees in South America.

Results from reciprocal F_1 crosses indicate that bee size is a function of egg genotype, comb cell size and maternal genotype. The importance of maternal inheritance for reducing worker bee size variation within a colony is discussed. Advantages of smaller worker bee size are evaluated for Africanized bees.

There are no effective reproductive isolating mechanisms operating between Africanized and European honey bee populations. Both Africanized and European queens mated with equal success with Africanized drones as measured by the numbers of spermatozoa in the spermatheca. The potential impact of Africanized bees on North America is analyzed with respect to hybridization and genetic introgression, resource competition, and selection advantages for European bees in temperate regions.

CHAPTER I
INTRODUCTION

Evolutionary Origin and Distribution of Honey Bees

Honey bees of the genus Apis have their greatest diversity in Asia (Michener 1979). Earliest fossil evidence for the genus is from Oligocene deposits in Europe (Zeuner and Manning 1976). The evolutionary relationships of the four generally recognized species of Apis are reviewed by Michener (1974). Three of the species (A. cerana, A. dorsata and A. florea) are native only to Asia (Michener 1979; Ruttner 1975). The western honey bee (A. mellifera) is native to Africa, western Asia and Europe and may have evolved in tropical or subtropical Africa (Wilson 1971) or the Near East (Ruttner 1975). The widely different climatic conditions and floral resources under which populations of A. mellifera evolved have resulted in a number of geographically recognizable subspecies (Alpatov 1929, 1933; Br. Adam 1966; Dupraw 1965; Ruttner 1968, 1975, 1976a, 1976b; Smith 1961; Wafa, Rashad and Mazeed 1965).

Importation of African Honey Bees into Brazil and Their Dispersal Throughout South and Central America

European honey bees (A. mellifera mellifera and A. m. ligustica) had been introduced into Brazil by 1845 (Gerstaker cited in Pellet 1938; Woyke 1969). A. m. mellifera is native to Europe in the regions west and north of the Alps and extending east into Central Russia; A. m.

ligustica is native to the Italian peninsula (Ruttner 1975). Because these European honey bee populations were not very successful in tropical and subtropical habitats of Brazil (Michener 1972), researchers believed that they could improve Brazil's honey production by breeding a honey bee better adapted to local conditions (Woyke 1969). With this intention, honey bee queens from South Africa (A. m. scutellata, formerly classified as adansonii, see Ruttner 1976a, 1976b, 1981) were imported into southeastern Brazil in 1956 (Kerr 1967). The following year, swarms escaped and hybridized with established European honey bees. The descendents from this hybridization are known as Africanized honey bees (Goncalves 1982). Details of the introduction and subsequent spread throughout South America have been extensively reviewed (Goncalves 1974, 1975, 1982; Kerr 1967; Michener 1972, 1975; Taylor 1977, 1985; Taylor and Williamson 1975; Woyke 1969).

In the 30 years since African honey bees were imported into southeastern Brazil, their hybridized offspring have rapidly dispersed throughout tropical South and Central America and are now as far north as Honduras and El Salvador (Rinderer 1986). The dispersion from their original importation site into new areas has been rapid--200-500 km per year (Taylor 1977, 1985; Winston 1979a). As Africanized honey bees have colonized new areas, they have achieved dramatic population densities (Michener 1975). There are now probably more than ten million feral colonies in South and Central America (Winston, Taylor and Otis 1983). Their success in these new habitats, compared with the lack of success of European honey bee populations, may be attributed to their foraging behavior which is more suited to the resource patterns of the tropics (Nunez 1973, 1979a, 1982; Rinderer, Bolten, Collins and Harbo 1984;

Rinderer, Collins and Tucker 1985; Winston and Katz 1982). As a result of both foraging success and the length of time throughout the year that resources are available in the tropics, Africanized honey bees have a high annual reproductive rate, which is responsible for both their rate of dispersal into new areas and their high colony densities. Net reproductive rates for Africanized bees are estimated to be 16 colonies per colony per year based on demographic data collected in French Guiana (Otis 1980, 1982a), compared with 0.92-0.96 (Seeley 1978) or, when afterswarms are considered, 3-3.6 (Winston 1980a; Winston, Taylor and Otis 1983) colonies per colony per year for European honey bees in North America.

Characteristics of Africanized Honey Bees

The most well known characteristic that differentiates Africanized honey bees from European honey bees is their stinging behavior (Collins, Rinderer, Harbo and Bolten 1982; Stort 1974, 1975a, 1975b, 1975c, 1976). Because of their stinging behavior, Africanized bees are a health hazard for both humans and domestic animals (Taylor 1986). Collins, Rinderer, Harbo and Bolten (1982) compared the colony defense behavior of Africanized honey bees in Venezuela with European bees under identical conditions in Venezuela and with a population of European bees in Louisiana, U.S.A. Africanized honey bees responded more rapidly and in much greater numbers, resulting in 5.9 times as many stings in a target compared with European honey bees in Venezuela and 8.2 times as many stings compared with European bees in Louisiana. Two additional components of Africanized honey bee defense behavior increase their potential as a health hazard. Compared to European bees, Africanized bees pursue a source of disturbance for a greater distance (160 versus

22 meters) and remain disturbed for a greater period of time (28 versus 3 minutes) (Stort 1971 cited in Goncalves 1974). Differences in defense behavior between Africanized and European bees do not appear to be a function of either quantitative differences in pheromone production (Crewe and Hastings 1976) or numbers of olfactory structures on the antennae (Stort and Barelli 1981).

There is a difference in natural comb cell size between Africanized and European populations. The width between opposite sides of the hexagonal cells for the Africanized population in Brazil averaged 5.0 mm compared with 5.4 mm for the European population in Canada (Michener 1972). In a recent study, cells built by Africanized swarms in Venezuela were 4.8-4.9 mm wide and those built by European swarms in Louisiana, U.S.A. were 5.2-5.3 mm wide (Rinderer, Tucker and Collins 1982). Adult Africanized bees are smaller than European bees (62 mg compared with 93 mg, unengorged) (Otis 1982b; Otis, Winston and Taylor 1981). However, as Africanized honey bees disperse into areas with extensive European honey bee populations, size differences between the two populations may become less distinct. Increased hybridization between the two populations could result in bees with an Africanized genome developing in European comb cells, resulting in larger Africanized bees. Therefore, methods used to identify Africanized honey bees based on size parameters, for example, morphometric analysis, may become less reliable. As Daly, Hoelmer, Norman and Allen (1982) point out, there is a "difficulty in using phenotype characters to identify genetically different, but closely related populations" (p. 593). This will be more evident as Africanized honey bees disperse into areas of Central America and particularly Mexico, where large populations of

European honey bees exist. Factors determining honey bee size and potential problems of Africanized honey bee identification based on size are analyzed in Chapter III.

The cuticular hydrocarbon composition of Africanized honey bees is significantly different from that of European honey bees (Carlson and Bolten 1984). The differences are particularly striking for the 35, 37, 39, 41 and 43 carbon alkenes and alkadienes that total over 22% of the hydrocarbons extracted from Africanized bees but only 1-3% of the hydrocarbons extracted from European bees. Because hydrocarbon composition is not affected by honey bee size or diet, using hydrocarbon analysis to distinguish between Africanized and European honey bees has great potential. However, further research to determine heritability patterns for different hydrocarbon components is needed.

Differences between Africanized and European honey bees have also been demonstrated for foraging behavior (Nunez 1973, 1979a, 1982; Rinderer, Bolten, Collins and Harbo 1984; Rinderer, Collins and Tucker 1985; Winston and Katz 1982), egg development times (Harbo, Bolten, Rinderer and Collins 1981), selection preferences for nest cavity sizes (Fletcher 1976; Michener 1972; Rinderer, Collins, Bolten and Harbo 1981; Rinderer, Tucker and Collins 1982), hoarding behavior (Rinderer, Bolten, Harbo and Collins 1982), worker bee longevity (Winston and Katz 1981), morphometric analysis (Daly and Balling 1978), and allozyme patterns (Nunamaker and Wilson 1981; Sylvester 1982). Africanized honey bee populations in South America are reported to have a high colony reproductive (swarming) rate compared with European honey bee populations in North America (Otis 1980, 1982a; Winston 1979b, 1980a; Winston, Dropkin and Taylor 1981; Winston, Taylor and Otis 1983).

However, those investigations have not been conducted under similar environmental or experimental conditions. Therefore, comparisons of reproductive rates between Africanized and European honey bees using those data are inappropriate for either identifying differences in reproductive rates for tropical and temperate honey bee populations or for identifying factors responsible for the success of Africanized bees in tropical regions.

Purpose of My Research

African and European honey bee populations evolved under very different resource and climatic conditions. The presence of both Africanized and European honey bees in Venezuela provided the opportunity to study both populations under identical conditions in the tropics. Differences between the two honey bee populations that make Africanized bees more successful in tropical regions could then be evaluated. The underlying assumption of my research was that the life history of Africanized honey bee populations in South America (as well as the parental population in Africa) is characterized by a high reproductive rate. Demographic features expected to be correlated with this high rate of colony reproduction include short worker bee development times, small worker bee size, rapid queen development and maturation, and increased egg laying and brood production rates. Predictions involving these demographic characteristics led to a series of experiments that are presented and discussed in the following chapters.

In addition, the question of reproductive isolation versus hybridization and differential selection between the two populations in tropical conditions was experimentally evaluated. Whether there is

hybridization or reproductive isolation between Africanized and European honey bee populations could result in very different scenarios for the potential impact of Africanized honey bees on North America, particularly the U.S.A.

Identification of Honey Bees Used in My Research

For the experiments presented here, Africanized honey bee colonies were established from queens removed from feral colonies in an area in eastern Venezuela where there were no known European honey bees. They were identified as Africanized bees primarily by their distinctly smaller comb cell size as compared with European honey bees.

European honey bees used in the experiments were from commercially produced queens from three different queen breeders in the U.S.A. Additional European lines were obtained from the U.S. Department of Agriculture Bee Research Laboratories in Madison, Wisconsin, and Baton Rouge, Louisiana. All of these European queens were either naturally mated or instrumentally inseminated in the U.S.A. and then shipped to Venezuela.

When and Where Research Was Conducted

All field research with Africanized and European honey bees was conducted from December 1978 through February 1980 at the Ministerio de Agricultura y Cria de Venezuela Africanized Honey Bee Research facilities near Maturin, Monagas. The area originally was a Tropical Dry Forest [sensu Holdridge Life Zone System (Holdridge 1964; Ewel and Madriz 1968)]. The forest had been partially cleared, and the area was grazed by cattle.

All Africanized and European honey bee comparisons were made at the same time under identical experimental conditions. Field and experimental methods are described for each of the experiments in the appropriate chapters.

A few experiments with European honey bees only were conducted in the U.S.A. to confirm techniques developed and used while in Venezuela. These experiments were undertaken either at the U.S. Department of Agriculture Bee Breeding and Stock Center Laboratory in Baton Rouge, Louisiana, or at the bee research facilities of the Institute of Food and Agricultural Sciences at the University of Florida, Gainesville.

CHAPTER II
WORKER BEE DEVELOPMENT TIMES

Introduction

The presence of both Africanized and European honey bees, Apis mellifera, in South America allows for comparisons to be made under identical conditions between a population that has evolved in the tropics and one that has evolved in temperate regions. Africanized honey bee populations in South America are reported to have a high colony reproductive (swarming) rate compared with European honey bee populations in North America (Otis 1980, 1982a; Seeley 1978; Winston 1979b, 1980a; Winston, Dropkin and Taylor 1981; Winston, Taylor and Otis 1983). However, these investigations have not been conducted under similar environmental or experimental conditions. Therefore, comparisons of reproductive rates between Africanized and European honey bees using these data are inappropriate either for identifying differences in reproductive rates between tropical and temperate honey bee populations or for identifying factors responsible for the success of Africanized bees in tropical regions.

Reproductive rates in honey bees are a function of colony growth rates which are a result of an interaction of at least three factors: resource availability, resource utilization efficiency (foraging success, brood production efficiency, and bee size), and colony demographic parameters. Worker bee longevity is the only demographic

parameter that has been compared between Africanized and European bees under identical conditions. The greater longevity of European honey bees (Winston and Katz 1981) gives European bees a colony growth rate advantage. Other demographic characteristics that affect reproductive rates of Africanized and European honey bees (for example, worker bee development times, brood mortality, queen development and maturation periods, queen fecundity and brood production rates) have not been evaluated for Africanized and European bees under similar conditions. As part of a larger study evaluating these demographic parameters, this study compares worker bee development periods for Africanized and European honey bees in Venezuela.

Smith (1958a) and Tribe and Fletcher (1977) reported that the total development period (from oviposition to adult emergence) for worker bees of Apis mellifera adansonii (now classified as A. m. scutellata; Ruttner 1976a, 1976b, 1981) from South Africa was between 18.6-20 days. Similar development times for the Africanized honey bee populations (descendents of A. m. scutellata) in Brazil have been presented (Kerr, Goncalves, Blotta and Maciel 1972; Wiese 1972). Worker bee development times for European populations (primarily A. m. mellifera, ligustica, carnica and caucasica) from Europe and North America range from 20-24 days (Jay 1963).

The differences in development times between African (and Africanized) and European genotypes, which range from 1.4 to 5.4 days, are difficult to evaluate because they are based on data collected under very different experimental conditions. Jay (1963) summarized a number of factors that affect development times: seasonal variation in temperature; temperature differences in different areas within the brood

nest; colony size, which affects both brood nest temperature and feeding frequency and quality; and nectar and pollen resources, which also affect feeding quantity and quality. Valid comparison of development times between genotypes or populations can only be made when these factors are controlled under similar experimental conditions.

The importance of slight temperature differences on development time cannot be overstated. Development times for European worker bees in Wisconsin, U.S.A., averaged 20.5 days but ranged from 20-24 days, depending on differences in temperature in different areas of the brood nest (Milum 1930). Harbo and Bolten (1981) showed that fertilized eggs kept at 34.8°C hatched about 1.4 hours sooner than those kept at 34.3°C. This difference in egg hatch time for only a 0.5°C difference in temperature can be extrapolated to approximately 10 hours for the entire development period [calculated from Harbo and Bolten (1981)]. However, normal temperatures within a brood nest can vary to a much greater extent (Milum 1930; Jay 1963). For example, when Tribe and Fletcher (1977) determined the development rates of African honey bees in South Africa, they recorded that temperatures in the brood nest varied from 26-34°C.

In an incubator with controlled temperature and humidity, eggs from Africanized genotypes hatched significantly sooner than eggs from European genotypes, 69.6 $\pm$ 1.06 hours compared with 73.3 $\pm$ 1.14 hours (Harbo, Bolten, Rinderer and Collins 1981). Egg development requires that only temperature and humidity be controlled and can therefore be evaluated independently of colony-level parameters. However, differences between Africanized and European genotypes for total worker bee development periods need to be evaluated within a colony in order to

allow for normal feeding and growth. Worker bee development rates are a result of an interaction between the egg genotype and the colony. There are three colony-level factors that need to be considered when comparing total development time of Africanized and European worker bees.

First is the effect of comb cell size. There is a difference in natural comb cell size between Africanized and European populations. The width between opposite sides of the hexagonal cells for the African population in Africa measured 4.77-4.94 mm (Smith 1958a). Cells for the Africanized population in Brazil averaged 5.0 mm (range 4.8-5.4 mm) (Michener 1972), but cells of the Africanized population in Venezuela averaged 4.8 mm (range 4.5-5.0 mm) (Chapter III; Rinderer, Tucker and Collins 1982). Cells from the European population from Ontario, Canada, averaged 5.4 mm (range 5.2-5.7 mm) (Michener 1972), and those from Louisiana, U.S.A., averaged 5.2-5.3 mm (range 5.2-5.4 mm) (Rinderer, Tucker and Collins 1982). Adult bee size is a function of comb cell size (Grout 1937); adult Africanized bees are smaller than European bees (62 mg compared with 93 mg, unengorged) (Otis 1982b; Otis, Winston and Taylor 1981).

Abdellatif (1965) suggested that larvae in smaller comb cells received less food which caused them to elongate and become sealed earlier. Also, Tribe and Fletcher (1977) suggested that the difference in development time for African and European genotypes may be a function of the small African bee size. Therefore, the effect of comb cell size needs to be considered when comparing development times of Africanized and European honey bees.

The second colony-level factor is the effect of nurse bee genotype. There may be behavioral differences and/or physiological differences in

the way in which nurse bees from the two populations interact with the developing larvae. Mel'nichenko (1962) suggested that differences between nurse bee genotypes might affect developmental rates as well as size of developing larvae. For European honey bees, Lindauer (1953) calculated that each developing larva requires over 2785 adult bee visits taking a total of 10.3 hours. This appears to provide sufficient opportunity for possible genotype differences, either quantitative or qualitative, to affect development rates. In addition to potential qualitative or quantitative differences in feeding of larvae, nurse bees of different genotypes may also maintain different brood nest temperatures. Therefore, development times for Africanized and European worker bees were evaluated in both Africanized and European colonies.

The third colony-level factor affecting worker development is colony size (number of worker bees in a colony). Colony size affects both brood nest temperature and larval feeding rates, which, as already discussed, are two major factors affecting development times.

In addition to these colony-level parameters, resource conditions also affect development time. Nelson and Sturtevant (1924) reported that development of European bees was more rapid with increased larval feeding associated with a nectar flow. Ribbands (1953) and Jay (1963) both summarized evidence of the effect of food on larval development rates. Therefore, all comparisons of worker development times were conducted simultaneously to avoid any differences due to resource conditions.

This paper reports the results from a comparison of the development times of Africanized and European honey bees under identical conditions in Venezuela. The experimental design allowed for the discrimination

between the effects of egg genotype and the colony-level parameters of comb cell size and nurse bee genotype on worker bee development time. These experiments were conducted during July-October 1979.

Methods

Table 2-1 summarizes the experimental design. Four experimental colony treatments were established as follows:

i. Africanized comb cell size, Africanized nurse bees (A55)

ii. Africanized comb cell size, European nurse bees (H2)

iii. European comb cell size, Africanized nurse bees (A41)

iv. European comb cell size, European nurse bees (IBR877).

Each experimental colony was a five-frame hive (22 liters) with four empty combs and one comb with honey and pollen and approximately 2 kg of young adult bees (Africanized or European, depending upon treatment). Because natural nectar and pollen resources were available irregularly throughout the experimental period (16 weeks), the colonies were supplemented with honey and pollen as necessary.

European comb was built from commercially-produced beeswax foundation that had been fastened into standard wooden frames. Africanized comb was naturally built (not from foundation) by Africanized bees in empty standard wooden frames to facilitate manipulation and colony inspection.

The queens in colonies with Africanized nurse bees (A55 and A41) were Africanized queens produced by standard queen rearing methods (Laidlaw 1979) and then naturally mated to Africanized drones. Mating occurred in an area of eastern Venezuela that had a large feral population of Africanized honey bees with no known European honey bees present (near San Jose de Buja, Monagas, Venezuela). The feral colonies

from which the Africanized queen mothers were extracted were also from this area. The colonies were identified as Africanized honey bees by both their behavior and their small comb cell size characteristic of the Africanized population (4.5-5.0 mm, see Chapter III).

The queens in the colonies with European nurse bees (H2 and IBR877) were European queens that had been mated to European drones in the U.S.A. and transported to Venezuela. Line H2 was from a commerical queen producer in the southeastern U.S.A.; IBR877 was an outbreed line from the U.S. Department of Agriculture Bee Breeding and Stock Center Laboratory in Baton Rouge, Louisiana, U.S.A.

The source for the Africanized egg genotype (A26) was a queen removed from a feral colony of Africanized honey bees in the San Jose de Buja area. The colony was identified as Africanized by its behavior and characteristic comb cell size. The source of the European egg genotype (Y5) was a queen commercially produced in the southeastern U.S.A. and shipped to Venezuela.

Because adult longevity is from 2 to 5 weeks for European honey bees (Woyke 1984) and 2 to 3 weeks (or less) for Africanized honey bees (Winston 1979b; Winston and Katz 1981), experimental colonies were established 10 weeks prior to the start of the experiment. This was sufficient time to insure that at the beginning of the developmental trial all the adult bees present within the experimental colonies had developed in those colonies, and, therefore, were offspring of a known genetic line having developed within a known comb cell size. Before development times were measured, the worker bee populations in the experimental colonies were equalized as much as possible by removing random samples of bees from the most populous colonies.

The experimental colonies were placed in an apiary under a roof with completely open sides. The roof served two purposes. First, the colonies were in complete shade, which reduced any effects from differences in ambient temperature and sunlight. Second, colonies could be opened and inspected in order to monitor development with a minimum of disturbance, especially during rain.

Eggs were collected from queens of the two designated egg source lines (A26 and Y5), that were established in modified colonies similar to those used by queen producers in the U.S.A. (Harp 1973). These colonies consisted of five standard frames with the middle frame isolated from the four others by queen excluder side and top panels. The excluder panels have a mesh size that restricts the queen from passing through because of her wider thorax, but allows worker bees to pass through to feed and communicate with the queen. Thus, the queen was isolated on a specific comb so that eggs could be collected that would then be placed into one of the four experimental colonies to evaluate development time.

The comb used had the appropriate comb cell size for the experimental colony into which it would be placed. Comb cell size was measured in each experimental nurse bee colony and for each Africanized and European egg comb put into each experimental colony.

The queens were caged on each comb for 24 hours so that a large, uniform egg sample could be collected. Eggs were monitored only from the center of each frame, which insured a more uniform temperature during development as well as uniform brood nest position. The large egg sample also insured that the monitored eggs were in a normal environment, surrounded by similarly-aged developing bees. The thirty

or forty eggs selected to be monitored for development formed either a 3x10 or a 4x10 cell area. A reference point which facilitated locating the designated development sample was indicated by colored pins inserted five cells to the left of each original egg row.

The two combs (one comb from each egg source) were placed in the center of each experimental colony at the same time. The cells with the eggs to be monitored faced each other in order to reduce any effects of brood nest position. Two frames of brood were removed from each experimental colony to make room for the experimental frames. This also reduced the amount of brood being reared in each experimental colony, insuring that the monitored eggs would be optimally fed.

The pairs of frames were put into the four experimental colonies on four successive days because of the 24 hours needed to collect each set of eggs. Once the eggs were put into the experimental colonies, they were inspected every day at 0800 hours. The survivorship and developmental status of each original test group egg was recorded.

The sample size of eggs monitored was selected to minimize the time each colony would need to be opened for observation in order to minimize disturbance. When a colony was disturbed, bees flew from the comb and ran on the bottom of the hive, resulting in temperature fluctuations and interrupted feeding of larvae. Colonies were carefully opened, using minimal amounts of smoke. Adult bees were not shaken off the combs but rather gently pushed aside to observe the development stage within the cells. Inspections more frequent than every 24 hours also increased the level of disturbance, especially in the Africanized colonies (A55 and A41). The advantage of more frequent monitoring to more accurately

record time of developmental changes was outweighed by the negative effects of disturbance on development rates.

Temperature of the brood nest in the space between the two monitored combs was recorded periodically by placing a thermometer into that area through a hole in the hive cover. This was only an approximate measure of temperature because development occurs within the cells where temperature is less affected by the ventilating air currents within the hive.

Results

Table 2-2 presents the development times for unsealed brood, sealed brood, and total development times from oviposition to adult emergence for Africanized and European egg genotypes in each of the four experimental treatments. Table 2-3 summarizes the argumentation for evaluating the interactions of egg genotypes with the colony-level parameters of comb cell size and nurse bee genotype on worker bee development times. The best tests to use to compare differences between the Africanized and European egg genotypes are evaluating pairs in each of the four experimental nurse bee colonies (A55, H2, A41, and IBR877), i.e., AxB, CxD, ExF, and GxH. In these comparisons, the colony-level parameters (colony population, position within the brood nest, temperature, comb cell size, and nurse bee genotype) are identical and allow for only differences in egg genotype to be compared. Tables 2-4, 2-5 and 2-6 summarize the results from the statistical analyses for the unsealed brood, sealed brood, and total development times, respectively. Data were analyzed with the Kolmogorov-Smirnov one-tailed test using the chi square distribution, df = 2 (Siegel 1956).

Africanized worker bees developed faster than European bees (ACEG x BDFH). The unsealed larval period was 4.3 ± 0.4 days compared with 4.9 ± 0.4 days, $P<0.001$; the sealed larval and pupal period was 11.6 ± 0.5 days compared with 11.9 ± 0.4 days, $P<0.01$; and the total development time was 18.9 ± 0.3 days compared with 19.8 ± 0.4 days, $P<0.001$. There was no significant effect of comb cell size or nurse bee genotype on development times. These differences in total development time are similar to the differences found between three different lines of Africanized and three different lines of European honey bees compared in another study (19.2 days compared with 20.0 days, Table A-1).

Comb cell sizes for the experimental colonies and egg sample frames are presented in Table 2-7. Temperatures recorded for all experimental colonies varied from 35-36°C.

When differences in development times between Africanized and European egg genotypes were compared for each stage of development, the greatest difference was observed in the unsealed larval stage (Table 2-8). However, this was not a result of a differential acceleration of development during the unsealed larval period for the Africanized honey bees. The proportion of unsealed larval development time to total development time and the proportion of sealed brood development time to total development time were compared for the Africanized and European honey bee populations following angular transformations of the proportions (Sokal and Rohlf 1969). These proportions were not significantly different between the Africanized and European honey bees.

The differences recorded for the unsealed brood stage between the Africanized and European honey bees may be an artifact of the experiment for two reasons. First, the 24-hour observation interval may obscure

exact timing of developmental changes. Second, the process of sealing is not a precise developmental stage and may take from six hours (Lindauer 1953) to 24 hours (Jay 1963). When the unsealed and sealed brood stages are combined, the proportional differences between the two populations are the same as for egg development times and total development times (Table 2-8).

Mortality for different developmental stages for each colony treatment is presented in Table 2-9 (see also Table A-2). Mortality was high (26-37%) for larvae in the experimental colony (H2) with European nurse bees on Africanized comb cell size. The high mortality during the larval stage may be a result of the reduced ability of larger European nurse bees to feed the developing larvae in smaller Africanized comb cells. There was also a high egg mortality recorded for European eggs in A41 and IBR877--34 and 70%, respectively. Woyke (1977) reports normal mortality may be as high as 10-50% depending on the season. Garofalo (1977) also reports varying mortalities depending on both the size of the colony and the time of year: eggs 10-25%, larvae 11-37%, pupae 5-7%, and all developmental stages combined 25-53%.

Discussion

This study is the first to evaluate worker bee development times between Africanized and European honey bees as an interaction between egg genotype and colony-level parameters. Differences in worker bee development times were independent of the colony-level parameters of comb cell size and nurse bee genotype but were dependent on egg genotype differences between the Africanized and European populations. The difference in development times between these two populations was not as

large as expected from previous reports, which underscores the importance of making comparisons under identical conditions.

The proportional difference (5.7%) in egg development times between Africanized (A26) and European (Y5) honey bees reported by Harbo, Bolten, Rinderer and Collins (1981) is identical to the proportional difference in total development time reported in the present study (5.7%, see Table 2-8). Egg development time is a function of the inherent rate characteristic of the particular genotype because colony-level parameters (e.g., feeding) are not involved (Harbo, Bolten, Rinderer and Collins 1981). Using egg development to evaluate differences in total development between genotypes (or populations) is advantageous because egg development times are easier to evaluate, take less time, have fewer variables to control (temperature and humidity only), and can be evaluated in an incubator rather than in a colony, avoiding problems associated with disturbing the colony during observations. It must be noted, however, that by using egg development times one can only extrapolate proportional differences between genotypes for total development time but cannot extrapolate the absolute total development time.

A prerequisite for high reproductive rates would be a rapid colony growth rate. However, the importance of worker development time to the rate of colony growth (increase in numbers of bees in a colony) has apparently been misunderstood, e.g., see Fletcher (1977a, 1978), Fletcher and Tribe (1977a), Tribe and Fletcher (1977), Winston (1979b), Winston, Dropkin and Taylor (1981), Winston and Katz (1982), Winston, Taylor and Otis (1983). The difference in worker development times observed for Africanized and European honey bees is not a factor

contributing to either differences in rate of colony population increase or to differences in reproductive rates between the two honey bee populations.

The importance attributed to worker development time on the rate of colony growth may be a result of confusing colony population increase (increase in the number of bees in the colony) with general population growth models designed for other species in which all individuals are potential reproductives. For honey bees, individual (or worker bee) development time is not equal to generation time. Organism growth models must be used to evaluate colony growth even though the number of individual worker bees within the hive increases. The hive is the organism. Worker bee development time does not affect the rate of colony growth. Worker development time affects only the length of time between a given change in egg laying rate and its resulting change in population increase or decrease. Africanized bees develop in 19 days and begin their population increase (=growth) on the 19th day of the colony cycle, compared with the 20th day for European bees. This difference is trivial compared to potential differences from other demographic factors that do affect rates of colony growth. Egg laying and brood production rates, worker bee longevity, brood mortality, and resource availability are factors that do affect the rate of colony population increase and, therefore, affect the reproductive rates.

Tribe and Fletcher (1977) have suggested that African worker bees have a shorter unsealed development stage because they do not grow as large as European honey bees. They compare their data for African bees with data for European bees in the literature and conclude that African bees have a 20-30% shorter unsealed larval stage. There are four

problems with their analysis. First, as already pointed out, using the duration of the unsealed stage has inherent problems because it is not a precise development stage. Second, comparisons based on data collected under different experimental conditions are not valid. Third, their logic is perhaps circular with respect to the question of larval size and larval development times. In the present study, development time was not size-related for either Africanized or European honey bees. Africanized honey bees that developed in European comb had the same development times as those that developed in Africanized comb even though Africanized bees reared in European comb were significantly larger (16%; Chapter III). The same relationship was true for European honey bees with a 17% increase in size of bees from European comb compared with bees from Africanized comb. And fourth, their comparison is in itself incorrect. Rather than compare the differences in unsealed development times between African and European populations to determine if the African population has a relatively shorter duration as unsealed larvae, they should have used the proportion of unsealed development period to total development period in order to compare African and European populations. In the present study, the relative times spent as an unsealed larvae to the total development time for both the Africanized and European genotypes were not significantly different. The differences in development time between Africanized and European populations appear constant throughout development without any developmental acceleration during the larval stage for either Africanized or European honey bees.

TABLE 2-1. Experimental matrix for evaluating interaction of egg genotype, comb cell size, and nurse bee genotype on worker development times. A - H represent each treatment.

	AFRICANIZED EGG GENOTYPE (A26)	EUROPEAN EGG GENOTYPE (Y5)
AFRICANIZED COMB CELLS		
AFRICANIZED NURSE BEES (A55)	A	B
EUROPEAN NURSE BEES (H2)	C	D
EUROPEAN COMB CELLS		
AFRICANIZED NURSE BEES (A41)	E	F
EUROPEAN NURSE BEES (IBR877)	G	H

TABLE 2-2. Interaction of egg genotype, comb cell size, and nurse bee genotype on worker bee development time (days): median, (range), mean ± SD, (n = sample size).

	AFRICANIZED EGG GENOTYPE (A26)		
	US[a]	SB[b]	TDT[c]
AFRICANIZED COMB CELLS			
AFRICANIZED NURSE BEES (A55)	4.0 (4-5) 4.2 ± 0.4 (n = 30)	12.0 (11-12) 11.6 ± 0.5 (n = 30)	19.0 (18-20) 18.8 ± 0.5 (n = 30)
EUROPEAN NURSE BEES (H2)	4.0 (4-5) 4.4 ± 0.5 (n = 29)	12.0 (11-12) 11.6 ± 0.5 (n = 29)	19.0 (19-20) 19.1 ± 0.2 (n = 29)
EUROPEAN COMB CELLS			
AFRICANIZED NURSE BEES (A41)	4.0 (4-5) 4.2 ± 0.4 (n = 30)	12.0 (11-12) 11.7 ± 0.5 (n = 30)	19.0 (18-19) 18.9 ± 0.2 (n = 30)
EUROPEAN NURSE BEES (IBR877)	4.0 (4-5) 4.3 ± 0.5 (n = 26)	12.0 (11-12) 11.7 ± 0.5 (n = 26)	19.0 (19) 19.0 ± 0 (n = 26)
TOTALS	4.0 (4-5) 4.3 ± 0.4 (n = 115)	12.0 (11-12) 11.6 ± 0.5 (n = 115)	19.0 (18-20) 18.9 ± 0.3 (n = 115)

[a]US = unsealed brood (unsealed larval development period only).
[b]SB = sealed brood (pre-pupae and pupae).
[c]TDT = total development time (oviposition to adult emergence).

TABLE 2-2--extended.

EUROPEAN EGG GENOTYPE (Y5)		
US	SB	TDT
5.0 (4-5) 4.9 ± 0.3 (n = 37)	12.0 (11-12) 11.8 ± 0.4 (n = 37)	20.0 (19-20) 19.6 ± 0.5 (n = 37)
5.0 (4-5) 4.9 ± 0.4 (n = 22)	12.0 (12) 12.0 ± 0 (n = 22)	20.0 (19-20) 19.9 ± 0.4 (n = 22)
5.0 (4-6) 5.0 ± 0.4 (n = 19)	12.0 (12-13) 12.0 ± 0.2 (n = 19)	20.0 (19-21) 20.0 ± 0.3 (n = 19)
5.0 (4-6) 4.7 ± 0.8 (n = 7)	12.0 (12-13) 12.1 ± 0.4 (n = 7)	20.0 (19-21) 19.8 ± 0.7 (n = 7)
5.0 (4-6) 4.9 ± 0.4 (n = 85)	12.0 (11-13) 11.9 ± 0.4 (n = 85)	20.0 (19-21) 19.8 ± 0.4 (n = 85)

TABLE 2-3. Summary of hypotheses and tests for evaluating development times; letters represent treatments (see Table 2-1).

H1: Worker bee development is faster for Africanized genotypes than for European genotypes.

A x B	Africanized comb cell size; Africanized nurse bees
C x D	Africanized comb cell size; European nurse bees
E x F	European comb cell size; Africanized nurse bees
G x H	European comb cell size; European nurse bees
A x H	Africanized comb cell size and nurse bees compared with European comb cell size and nurse bees
AC x BD	Africanized comb cell size; both nurse bee genotypes combined
EG x FH	European comb cell size; both nurse bee genotypes combined
AE x BF	Africanized nurse bees; both comb cell sizes combined
CG x DH	European nurse bees; both comb cell sizes combined
ACEG x BDFH	Both comb cell size and both nurse bee genotype variables combined

H2: Worker bee development is more rapid in Africanized comb cells than in European comb cells.

A x E	Africanized egg genotype; Africanized nurse bees
C x G	Africanized egg genotype; European nurse bees
B x F	European egg genotype; Africanized nurse bees
D x H	European egg genotype; European nurse bees
AC x EG	Africanized egg genotype; both nurse bee genotypes combined
BD x FH	European egg genotype; both nurse bee genotypes combined

H3: Worker bee development is more rapid with Africanized nurse bees than with European nurse bees.

A x C	Africanized egg genotype; Africanized comb cell size
E x G	Africanized egg genotype; European comb cell size
B x D	European egg genotype; Africanized comb cell size
F x H	European egg genotype; European comb cell size
AE x CG	Africanized egg genotype; both comb cell sizes combined
BF x DH	European egg genotype; both comb cell sizes combined

TABLE 2-3--continued.

H4: Worker bee development is more rapid with Africanized comb cells and Africanized nurse bees than with European comb cells and European nurse bees.

A x G	Africanized egg genotype
B x H	European egg genotype

TABLE 2-4. Unsealed brood development times. Hypotheses were tested using Kolmogorov-Smirnov one-tailed test, chi-square distribution, df = 2, alpha = 0.05 (Siegel 1956).

H1: Worker bee development is faster for Africanized genotypes than for European genotypes.

A X B	***[a]
C X D	**
E X F	***
G X H	NS
A X H	NS
AC X BD	***
EG X FH	***
AE X BF	***
CG X DH	***
ACEG X BDFH	***

H2: Worker bee development is more rapid in Africanized comb cells than in European comb cells.

A X E	NS
C X G	NS
B X F	NS
D X H	NS
AC X EG	NS
BD X FH	NS

H3: Worker bee development is more rapid with Africanized nurse bees than with European nurse bees.

A X C	NS
E X G	NS
B X D	NS
F X H	NS
AE X CG	NS
BF X DH	NS

H4: Worker bee development is more rapid with Africanized comb cells and Africanized nurse bees than with European comb cells and European nurse bees.

A X G	NS
B X H	NS

---

[a] ** = P<0.01
*** = P<0.001.
[b]Analysis may be NS because test used is conservative for small sample sizes using chi-square distribution.

TABLE 2-5. Sealed brood development times. Hypotheses were tested using Kolmogorov-Smirnov one-tailed test, chi-square distribution, df = 2, alpha = 0.05 (Siegel 1956).

H1: Worker bee development is faster for Africanized genotypes than for European genotypes.

A X B	NS
C X D	*[a]
E X F	NS
G X H	NS
A X H	NS
AC X BD	*
EG X FH	*
AE X BF	*
CG X DH	**
ACEG X BDF	**

H2: Worker bee development is more rapid in Africanized comb cells than in European comb cells.

A X E	NS
C X G	NS
B X F	NS
D X H	NS
AC X EG	NS
BD X FH	NS

H3: Worker bee development is more rapid with Africanized nurse bees than with European nurse bees

A X C	NS
E X G	NS
B X D	NS
F X H	NS
AE X CG	NS
BF X DH	NS

H4: Worker bee development is more rapid with Africanized comb cells and Africanized nurse bees than with European comb cells and European nurse bees.

A X G	NS
B X H	NS

[a] * = P<0.05
** = P<0.01.

TABLE 2-6. Total worker bee development times. Hypotheses were tested using Kolmogorov-Smirnov one-tailed test, chi-square distribution, df = 2, alpha = 0.05 (Siegel 1956).

H1: Worker bee development is faster for Africanized genotypes than for European genotypes.

A X B	***[a]
C X D	***
E X F	***
G X H	***
A X H	**
AC X BD	***
EG X FH	***
AE X BF	***
CG X DH	***
ACEG X BDFH	***

H2: Worker bee development is more rapid in Africanized comb cells than in European comb cells.

A X E	NS
C X G	NS
B X F	NS
D X H	NS
AC X EG	NS
BD X FH	NS

H3: Worker bee development is more rapid with Africanized nurse bees than with European nurse bees

A X C	NS
E X G	NS
B X D	NS
F X H	NS
AE X CG	NS
BF X DH	NS

H4: Worker bee development is more rapid with Africanized comb cells and Africanized nurse bees than with European comb cells and European nurse bees.

A X G	NS
B X H	NS

[a] ** = P<0.01
*** = P<0.001.

TABLE 2-7. Comb cell size for worker development time experiment: comb measurements = mm for 10 consecutive, horizontal cells, mean ± SD, (sample size).

	COMB CELL SIZE		
	NURSE BEE COLONY	AFRICANIZED EGG GENOTYPE	EUROPEAN EGG GENOTYPE
AFRICANIZED COMB CELL SIZE[a]			
AFRICANIZED NURSE BEES (A55)	47.5 ± 0.58 (4)	49.8 ± 0.50 (4)	45.8 ± 0.50 (4)
EUROPEAN NURSE BEES (H2)	48.2 ± 0.96 (4)	48.5 ± 0.58 (4)	48.5 ± 0.58 (4)
EUROPEAN COMB CELL SIZE[b]			
AFRICANIZED NURSE BEES (A41)	54.0 ± 0.0 (3)	54.0 ± 0.0 (3)	54.0 ± 0.0 (3)
EUROPEAN NURSE BEES (IBR877)	53.3 ± 0.58 (3)	53.3 ± 0.58 (3)	53.7 ± 0.58 (3)

[a]Natural comb built without foundation.
[b]Built from foundation.

TABLE 2-8. Comparison of differences in development times (in days) for Africanized and European honey bees for different developmental stages.

	DEVELOPMENTAL STAGES				
	EGG HRS (DAYS)[a]	UNSEALED BROOD[b]	SEALED BROOD[c]	UNSEALED & SEALED	TOTAL DEVELOPMENT
AFRICANIZED EGG GENOTYPE (A26)	69.6 (2.90)	4.3	11.6	15.9	18.80
EUROPEAN EGG GENOTYPE (Y5)	73.6 (3.07)	4.9	11.9	16.8	19.87
% DIFFERENCE[d]	5.7	14.0	2.6	5.7	5.7

[a]From Harbo, Bolten, Rinderer and Collins (1981); data used are their Africanized #3 = A26 and their European #5 = Y5.
[b]Unsealed larval period only.
[c]Pre-pupae and pupae.
[d]% Difference = [(Y5)-(A26)/(A26)] x 100.

TABLE 2-9. Mortality during different developmental stages.

	AFRICANIZED EGG GENOTYPE (A26)						EUROPEAN EGG GENOTYPE (Y5)					
	E_1[a]	E_2[b]	L_1[c]	L_2[d]	SB[e]	N[f]	E_1	E_2	L_1	L_2	SB	N
AFRICANIZED COMB CELL SIZE												
AFRICANIZED NURSE BEES												
A55	1	5	0	3	1	40	1	0	0	2	0	40
EUROPEAN NURSE BEES												
H2	1	0	3	7	0	40	1	0	0	13	0	36
EUROPEAN COMB CELL SIZE												
AFRICANIZED NURSE BEES												
A41	0	0	0	0	0	30	8	2	0	0	0	29
EUROPEAN NURSE BEES												
IBR877	0	0	2	2	0	30	13	6	1	0	0	27

[a]Mortality during first 24 hours in test colony (acceptance).
[b]Mortality between 24-72 hours (before hatching).
[c]Mortality between 72-96 hours (at time of hatching).
[d]Mortality during older larval stages, before sealing.
[e]Mortality during the pupal stage.
[f]N = total eggs monitored.

CHAPTER III
INTERACTION OF MATERNAL GENOTYPE, EGG GENOTYPE AND COMB CELL SIZE ON HONEY BEE WORKER SIZE AND SIZE VARIATION

Introduction

In the evolution of eusociality in bees (Apoidea), there is a considerable decrease in size variation of the workers within a colony. Worker size variation within a colony of primitively eusocial sweat bees (Halictidae) or bumble bees (Apidae) is much greater than the size variation of workers within colonies of highly eusocial stingless bees (Meliponinae: Melipona and Trigona) or honey bees (Apinae: Apis) (Brian 1952; Kerr and Hebling 1964; Medler 1965; Michener 1974). For example, the coefficient of variation (CV) for worker weights in a bumble bee colony may be as high as 31-37% (calculated from Brian 1952 for Bombus agrorum, Table B-1) whereas the CV for worker weights within a honey bee (Apis mellifera) colony is only 4-7% (Table B-2).

An effect of the reduction of size variation is that the mechanism for the division of labor of workers within a colony shifts from being size dependent to primarily age dependent (Michener 1974). In the primitively eusocial bumble bees, division of labor is size related (Brian 1952); large workers may be twice the size (linear measurements) of small workers within the same colony (Medler 1965). In highly eusocial stingless bees and honey bees, division of labor is primarily age dependent (Free 1965; Gary 1975; Kerr and Hebling 1964; Lindauer 1953; Seeley 1982). In honey bees, the workers proceed through a series

of age-related tasks. However, the sequence and duration of the different stages are flexible and depend on the needs of the colony.

An advantage of worker size variation within bumble bee colonies may be efficient utilization of diverse nectar and pollen resources that may be size dependent. Different sized workers within a colony specialize on those resources that they can most efficiently exploit (Heinrich 1979a). However, highly eusocial bees are not at a disadvantage with respect to resource utilization because they have evolved complex communication systems that allow foragers to monitor changing nectar conditions and to recruit workers from the colony to a particular resource. Therefore, both the species characterized by workers of highly variable sizes and those species characterized by uniformly-sized workers have evolved behaviors that enhance the efficiency of nectar and pollen exploitation.

The difference in intra-colony worker size variation between primitively eusocial and highly eusocial species of bees is so significant that Kerr and Hebling postulated that "some controlling mechanism leads to reduced variances among mature workers [Meliponinae and Apis], which are therefore of relatively uniform size" (1964, p. 267). Waddington (1981) hypothesized that the evolution and maintenance of the complex communication systems in Apis, Trigona and Melipona depend upon uniformity of worker bee size within a colony. Differences in bee size may result in miscommunication because resource "profitability" may be size dependent. For example, a high quality resource for a small bee may not be a high quality resource for a larger bee. However, a regulatory mechanism for reduced size variation has not been identified.

Honey bee size and size variation is a result of both genetic and environmental factors. Research has focused primarily on the extrinsic factors that affect bee size (e.g., comb cell size, nutrition and temperature). Honey bee worker sizes and honey bee comb cell sizes have been shown to be inter-related: because of the manner by which comb cells are constructed (Darwin 1859/1958), worker body size affects the diameter of cells they construct (Baudoux 1933; Glushkov 1958), and worker bee size is correlated to the size of cells in which they are reared (Baudoux 1933; Buchner 1955; Glushkov 1958; Grout 1937; Michailov 1927-28 cited in Alpatov 1929; Tuenin 1927).

This interaction between comb cell size and egg genotype may at first appear to provide a mechanism for both regulating bee size and reducing size variation among bees within a colony. However, the comb cell itself can become a source of variation in bee size. Although comb cell size appears quite uniform, especially when first constructed, the cells become variable in size as the number of generations reared in them increases, because pupal cocoons adhere to the cell walls, reducing cell diameter (Abdellatif 1965; Alpatov 1929; Buchner 1955; Grout 1937). For example, there is a 25% reduction in cell volume between cells from new and old combs (Table B-3). Comb cell volume has a greater variance than cell diameter and is not correlated with diameter (Table B-3).

In addition to comb cell size, there are other extrinsic factors that affect development and resultant bee size, e.g., quantity and quality of larval food, and temperature and humidity at which the larvae and pupae are reared (Buchner 1955; Fyg 1959; Jay 1963; Kulzhinskaya 1956; Michailov 1927-28 cited in Alpatov 1929). These same factors not only affect absolute size but are sources of size variation.

The importance of the genetic component to bee size can be inferred from the fact that different geographic populations of honey bees differ with respect to worker bee size (Alpatov 1929; Ruttner 1968, 1975, 1976a, 1976b; Wafa, Rashad and Mazeed 1965). Because honey bee queens mate with many different drones (Adams, Rothman, Kerr and Paulino 1977; Peer 1956; Roberts 1944; Taber 1954; Taber and Wendel 1958), the genetic component becomes an additional factor affecting size variation.

Africanized honey bees in Venezuela (descendents of A. m. scutellata) were smaller and had a smaller comb cell diameter (mean 4.8 mm between opposite sides of the hexagonal cells in the comb) compared with European bees in Venezuela (mean 5.4 mm) (Tables B-3 and B-4; Rinderer, Tucker and Collins 1982). An opportunity, therefore, existed to experimentally evaluate the interaction of both genotype and comb cell size on resultant worker bee size and size variation by studying both the Africanized and European honey bee populations under identical experimental conditions. The results from this study provide information not only on the proximal question involving the factors affecting bee size but also provide a mechanism by which size variation may be reduced within a honey bee colony.

Methods

Nine genotypes were evaluated: three Africanized, three European, and three F_1 reciprocal hybrids. The Africanized genotypes (A26, A57, and B39) were established from queens removed from feral colonies located in an area in eastern Venezuela with no known European honey bees. They were identified as Africanized honey bees by their comb cell sizes which were significantly smaller than European comb cell sizes (Tables B-3 and B-4; Michener 1972, 1975; Rinderer, Tucker and Collins

1982). The European genotypes (YD28 and WE1) were imported into Venezuela from the U.S. Department of Agriculture Bee Breeding and Stock Center Laboratory in Baton Rouge, Louisiana, U.S.A., and from a commercial queen producer from southeastern U.S.A., respectively. Queen YD28 was artificially inseminated with the spermatozoa from one drone; queen WE1 was naturally mated. The third European genotype (SDY1) was a daughter from line YK produced by another commercial queen producer from southeastern U.S.A. and artificially inseminated in Venezuela with a single drone from the same commercial line.

Two reciprocal hybrid lines were established from artificially reared queens (Laidlaw 1979) that were instrumentally inseminated with spermatozoa from single drones: Africanized queen x European drone (SDA12) and European queen x Africanized drone (SDY10 and SDY11). The Africanized queen and drone source was A26. The European queen and drone source was line YK. The hybrid lines were therefore genetically similar, but were the reciprocal of each other with respect to their queen and drone sources.

Queens were produced by the standard method of transferring young larvae from the desired queen line into artificial queen cells which were then introduced into cell-producing colonies (Laidlaw 1979). Mature queen cells were put into an incubator ($35 \pm 1^{o}C$) 72 hours prior to adult emergence. Newly emerged virgins were marked for individual identification and then put into individual cages and maintained in a strong, queenless colony for approximately one week until they were artificially inseminated.

Drones for instrumental inseminations were produced by caging drone comb containing sealed drone pupae from the desired drone source lines.

As drones emerged, they were placed into special holding cages and maintained in a colony so that worker bees could feed them until they matured. This manipulation insured that the drones used for inseminations were from the desired queen lines.

To collect eggs for the bee size experiments, queens from the nine genotypes were confined for five hours in their own colonies to a section of Africanized comb (mean cell size = 4.8 mm), using 8 x 8 cm push-in cages. These cages were made from 3 mm mesh hardware cloth and had queen excluder material soldered to the top to enable worker bees to pass through to tend the queen (Harbo, Bolten, Rinderer and Collins 1981). After five hours, the queens were removed from the combs. The 8 x 8 cm sections of comb with eggs from each queen were cut out and fitted into special frames. The nine sections were then placed in a strong Africanized colony (Africanized nurse bees and Africanized comb cell size) for development. The following day, eggs were collected in European combs (mean cell size = 5.4 mm) using the same procedure with the same nine queens except that the nine sections were put into a European colony (European nurse bees and European comb cell size) for development. Having all nine egg sources for each comb cell size treatment (Africanized or European) develop in the same colony controlled for additional variables affecting development and bee size: temperature and humidity, nurse bee genotypes and colony size (see Chapter II).

Fresh pupal weights were compared for each of the nine genotypes reared in both Africanized and European comb cell sizes. Pupal weights were measured on the 16th day after oviposition. This age corresponds to the period during pupal development of least weight change (Melampy

and Willis 1939). This was confirmed for fresh pupal weights by weighing a sample of pupae every 24 hours from day 11.5 post oviposition to 17.5 days post oviposition (Table B-5). Although Africanized bees develop one day faster than European bees (Chapter II), pupal weights can be compared because there is no significant difference in weights between adjacent days during this period of pupal development (Table B-5).

Pupal weights were used instead of adult weights in order to reduce variation resulting from differences in food engorgement and/or feces accumulation. Pupae were carefully removed from their comb cells by first removing the cappings and then spreading the cell walls with a forceps in order that the pupae could easily be removed without rupturing. Weights (to 1.0 mg) were recorded using Mettler Type H4 and H6 balances. Comb cell diameters were determined by measuring ten adjacent cells; three sets of measurements were made from each comb.

Results

Table 3-1 presents the experimental design matrix. The interaction of egg genotype and comb cell size on worker bee pupal weights for each of the nine genotypes is summarized in Table 3-2. Table 3-3 presents the results of the statistical analyses. When Africanized and European genotypes are reared simultaneously in the same colony (same comb cell size, nurse bee genotype, temperature and humidity, and colony size), the weights of the worker bees produced are different. Africanized bee pupae (111.1 $\pm$ 7.6 mg) that developed in Africanized comb cells were smaller than European bee pupae (123.3 $\pm$ 6.3 mg) that also developed in Africanized comb cells (ACE x MOQ, $P<0.001$). When worker bees of European genotypes are reared in Africanized comb cells, the cells are

sealed with strongly convex cappings similar to the way cells containing drones are sealed in order to accomodate their larger size. Africanized bee pupae (123.8 $\pm$ 6.2 mg) that developed in European comb cells were smaller than European bee pupae (139.5 $\pm$ 5.4 mg) that also developed in European comb cells (BDF x NPR, $P<0.001$). For each of the nine genotypes investigated, worker bee pupae that developed in Africanized comb cells were smaller than pupae that developed in European comb cells, $P<0.001$. There is a 43% increase in comb cell volume between Africanized and European combs (Table B-3), but the Africanized and European genotypes only increased in pupal weight by 11.4% and 13.1%, respectively (Table 3-2). These results show that both genotype and comb cell size affect worker bee size.

Table 3-4 presents the results for the pupal weights of the reciprocal F_1 hybrids and their respective maternal lines. Data from only European comb cells were used in order to observe genotype effects without the constraint of the small Africanized comb cells on European genotypes. Table 3-5 summarizes the results of the statistical analyses. The pupal weights of the hybrids from this reciprocal F_1 cross were significantly different from each other (H x J; H x L; $P<0.001$), but were the same as their respective maternal line (B x H; J x R; L x R).

Discussion

Reduction of Bee Size Variation

Bee size is a result of not only the interaction of egg genotype and comb cell size but also the maternal genotype. This can be seen by evaluating the reciprocal hybrid crosses. The genotype component for bee size is not a result of "simple" inheritance because pupal weights

of genetically similar, reciprocal F_1 hybrids are not the same. Because reciprocal F_1 hybrids are phenotypically different from each other, but phenotypically similar to their maternal lines, maternal genotype must interact with cell size and egg genotype to determine pupal weight. This is the first character in honey bees that has been shown to be influenced by maternal inheritance. Other genetic mechanisms cannot explain these results. The mechanism for maternal inheritance in worker size may be through egg size, which has been shown to be inherited (Roberts and Taber 1965; Taber and Roberts 1963).

Alles (1961) and Mel'nichenko (1962) suggested that differences between nurse bee genotypes might affect size of developing larvae. However, McGregor (1938) found that bee size was not affected by nurse bee genotype. In the experiments presented in this chapter, pupae from each of the genotypes were reared simultaneously in the same colony for each comb size treatment. Therefore, differences between the pupal weights of Africanized and European genotypes cannot be attributed to either nurse bee differences, cell size, or temperature but must be a result of both egg and maternal genotype differences.

The importance of maternal inheritance on bee size is that it reduces worker bee size variation within a colony. If maternal inheritance were not operating, worker bees of different sizes would be produced within a colony because of cell size differences and genotype differences. The effectiveness of maternal inheritance for reducing bee size variation can be demonstrated by comparing the degree of variation for the two parameters of bee size (comb cell volume and genotype) with the degree of worker bee size variation. Abdellatif (1965) showed that

when comb cell size variation increased 300%, bee size variation increased only 50%.

The genetic variation of worker bees within a colony is great because queens mate on the average with as many as 17 drones (Adams, Rothman, Kerr and Paulino 1977). There is some degree of mixing of spermatozoa in the spermatheca resulting in spermatozoa from at least 5 to 6 drones being used during one time interval (Page and Metcalf 1982). Evidence that maternal inheritance reduces size variation in genetically diverse worker offspring can be demonstrated by evaluating the size variation of offspring from single-drone and multiple-drone inseminated queens. The progeny of queens that were inseminated by spermatozoa from single drones (SDA12, SDY10, SDY11, SDY1 and YD28) were expected to be less variable than multiply-inseminated queens (A26, A57, B39 and WE1) because all eggs from the former queens would have been fertilized by a genetically identical male gamete. (Drones are haploid; all spermatozoa are produced by mitosis and are therefore genetically identical.) Evaluating the coefficient of variation (CV) for each treatment of genotype and comb cell size, there is no difference between the variation of progeny from single-drone inseminations versus those from multiple inseminations, as shown in Table 3-6 (Mann-Whitney U test, one-tailed, alpha = 0.05).

Additional evidence of maternal inheritance reducing size variation in genetically heterogeneous offspring comes from analyzing the results of the reciprocal F_1 cross. Because of the influence of maternal inheritance, subspecific differences in size between Africanized and European populations were not reflected in increased size variation of the hybrids compared with the parental types (Table 3-4).

Further evidence of the effectiveness of maternal inheritance reducing size variation can be seen by comparing the size variation within a colony to the size variation within a population. Alpatov (1929) found that within honey bee colonies, worker size variation (e.g., for tongue length) was less than the variation for the local population of a managed apiary. For seven different apiaries in Russia, each apiary had an average 22.3% (range 5-42%) increased variation over the mean colony variation within the apiary. Although the genetic homogeneity of the apiaries is artificially high as a result of management practices of the beekeepers compared with the variation of natural populations of animals (Alpatov 1929), within-colony variation was still noticeably reduced.

Evolution of complex communication systems in highly eusocial species may be responsible for selection for reduced size variation (Waddington 1981; Waddington, Herbst and Roubik 1986). Foragers within honey bee colonies have the ability to communicate information to nest mates about the direction, distance and "profitability" of new resources (von Frisch 1967) which may be interpreted correctly only if worker bees within the colony are the same size (Waddington 1981). Profitability of the resource may be size-dependent as Waddington (1981) suggested. That is, a high quality resource for a small bee may not be a high quality resource for a larger bee.

In addition to the profitablity component, correct interpretation of the distance component of the honey bee waggle dance (von Frisch 1967; Wenner 1962) may also be size-dependent. Different distance dialects occur not only between subspecies (Boch 1957; Gould 1982) but also between colonies (Esch 1978 cited in Gould 1982). There is greater

variation in individual dialects in colonies that are genetically heterogeneous compared with colonies that are genetically homogeneous (Gould 1982). Variation in bee size within a colony may accentuate differences in distance dialects and increase the possibility of miscommunication. Therefore, worker size variation within a colony of honey bees needs to be reduced in order for a communication system that recruits foragers to a particular floral resource to function correctly and efficiently with respect to either the profitability (Waddington 1981; Waddington, Herbst and Roubik 1986) or distance component. Maternal effects operate to reduce bee size variation within a colony of honey bees, thereby allowing their communication system to function effectively.

Africanized and European Honey Bee Size Difference

Several hypotheses have been suggested to explain the smaller worker bee size of the Africanized population. One advantage suggested for smaller size is more rapid development times, permitting more rapid colony growth resulting in increased reproductive swarming (Fletcher 1977a; Fletcher and Tribe 1977a; Tribe and Fletcher 1977). However, cell size and bee size do not affect development times, and, in addition, worker development times do not affect colony growth rates (Chapter II).

Fletcher and Tribe (1977a) and Tribe and Fletcher (1977) suggested that smaller bee size would permit greater numbers of worker bees to be reared on the same amount of food compared with larger bees. Advantages of increased worker numbers include frequency of reproductive swarming, colony defense and foraging success (Wilson 1971). Thus, smaller, individual bee size maximizes the use of the limited food that

characterizes the unreliable nectar availability in Africa (Tribe and Fletcher 1977). Smaller bee size increases the resource utilization efficiency of Africanized honey bees and may be a factor in the success and high reproductive rates of Africanized honey bees compared with European honey bees in tropical areas of South America (see Chapter VIII).

I suggest two other hypotheses to explain the advantages of smaller size in the Africanized population. First, smaller size is more efficient with respect to dissipating heat loads in tropical habitats (see also Heinrich 1979b). Fletcher (1978) reports that foraging may stop during the hottest part of the day, which would avoid the disadvantages of smaller size with respect to gaining a heat load. The sizes of two other subspecies of honey bees in Africa support this hypothesis. One of the smallest subspecies in Africa, A. m. litorea, is found in a very hot and dry area along the coast of Kenya and Tanzania. One of the largest subspecies, A. m. monticola, is found at higher elevations and colder temperatures on Mount Kenya.

Secondly, the advantage of smaller bee size may actually lie with the advantages of smaller cell size. For a given nest cavity volume, a larger number of worker bees can be produced if cell sizes are smaller. There is approximately a 25% increase in the number cells for a given comb area with smaller Africanized comb cells compared with larger European comb cells. Considering the advantages of increased worker numbers in a colony (Wilson 1971), the increase in worker numbers as a result of smaller cell size may be important, particularly if nest cavity volumes are limited.

Because maternal inheritance affects bee size, methods that use components of size to identify Africanized bees, e.g., morphometric analysis, may be invalid. Offspring from the cross of a European queen x Africanized drone (SDY10 and SDY11) are the same weight as offspring from European queen x European drone (SDY1) (Table 3-4). More importantly, the offspring from the cross of a European queen x Africanized drone are significantly different from offspring from Africanized queen x Africanized drone and Africanized queen x European drone matings. The European queen x Africanized drone mating represents the most probable scenario for initial hybridization in North America (see Chapter VII). That is, a virgin queen from a managed or a feral European colony mates with Africanized drones and produces offspring with a 50% Africanized genome. Analyzing the offspring using size as a component for identification may result in a false negative identification of Africanized bees. The extent of the problem would depend upon the degree to which particular linear measurements are either affected by maternal inheritance and/or are correlated with bee weight. As Daly, Hoelmer, Norman and Allen (1982) point out, there is a "difficulty in using phenotype characters to identify genetically different, but closely related populations" (p. 593).

TABLE 3-1. Effect of comb cell size and egg genotype on bee pupal weights. Experimental design matrix (code letters A-R used in tables of statistical analyses).

EGG GENOTYPES	COMB CELL SIZE	
	AFRICANIZED	EUROPEAN
AFRICANIZED QUEEN X AFRICANIZED DRONE		
A26[a]	A	B
A57[a]	C	D
B39[a]	E	F
AFRICANIZED QUEEN X EUROPEAN DRONE		
SDA12[b]	G	H
EUROPEAN QUEEN X AFRICANIZED DRONE		
SDY10[b]	I	J
SDY11[b]	K	L
EUROPEAN QUEEN X EUROPEAN DRONE		
YD28[b]	M	N
WE1[a]	O	P
SDY1[b]	Q	R

[a]Natural matings, multiple inseminations.
[b]Single drone insemination.

TABLE 3-2. Effect of comb cell size and egg genotype on bee pupal weights (mg). Means ± SD, (sample size).

EGG GENOTYPES	COMB CELL SIZE: AFRICANIZED[a]	COMB CELL SIZE: EUROPEAN[b]	% INCREASE
AFRICANIZED QUEEN X AFRICANIZED DRONE			
A26[c]	105.4 ± 4.6 (60)	121.7 ± 5.5 (80)	15.5
A57[c]	117.2 ± 6.8 (30)	128.6 ± 4.4 (40)	9.7
B39[c]	116.5 ± 3.6 (30)	123.1 ± 6.3 (40)	5.7
COMBINED	111.1 ± 7.6 (120)	123.8 ± 6.2 (160)	11.4
AFRICANIZED QUEEN X EUROPEAN DRONE			
SDA12[d]	112.9 ± 4.4 (30)	122.4 ± 4.4 (40)	8.4
EUROPEAN QUEEN X AFRICANIZED DRONE			
SDY10[d]	114.6 ± 3.7 (10)	133.7 ± 3.3 (23)	16.7
SDY11[d]	115.5 ± 3.2 (30)	138.9 ± 4.9 (30)	20.2
COMBINED	115.3 ± 3.3 (40)	136.7 ± 5.0 (53)	18.6
EUROPEAN QUEEN X EUROPEAN DRONE			
YD28[d]	126.8 ± 4.2 (30)	138.7 ± 3.5 (30)	9.4
WE1[c]	125.1 ± 4.3 (30)	143.3 ± 2.9 (30)	14.5
SDY1[d]	115.4 ± 4.2 (20)	135.0 ± 6.9 (19)	17.0
COMBINED	123.3 ± 6.3 (80)	139.5 ± 5.4 (79)	13.1

[a]Width between opposite sides of the hexagonal cell is 4.8 mm.
[b]Width between opposite sides of the hexagonal cell is 5.4 mm.
[c]Natural matings, multiple inseminations.
[d]Single drone insemination.

TABLE 3-3. Worker bee size: hypotheses and analyses (Mann-Whitney U test, one-tailed, alpha = 0.05). Letters refer to experimental treatments, see Table 3-1.

H1: Africanized bee pupae are smaller than European bee pupae independent of comb cell size.

ACE x MOQ	***[a]
BDF x NPR	***
ACE x NPR	***
BDF x MOQ	NS

H2: For a given egg genotype, pupae that develop in Africanized comb cell size are smaller than pupae that develop in European comb cell size.

A x B	***
C x D	***
E x F	***
G x H	***
I x J	***
K x L	***
M x N	***
O x P	***
Q x R	***
ACE x BDF	***
MOQ x NPR	***

[a] *** = P<0.001.

TABLE 3-4. Reciprocal F_1 cross. Pupal weights (mg), mean $\pm$ SD, (sample size). Data are from European comb cell size only.

AFR[a] QUEEN X AFR DRONE[b] (A26)	AFR QUEEN X EUR DRONE[c] (SDA12)	EUR[a] QUEEN X AFR DRONE[c] (SDY10)	EUR[a] QUEEN X AFR DRONE[c] (SDY11)	EUR QUEEN X EUR DRONE[c] (SDY1)
121.7 $\pm$ 5.5	122.4 $\pm$ 4.4	133.7 $\pm$ 3.3	138.9 $\pm$ 4.9	135.0 $\pm$ 6.9
(80)	(40)	(23)	(30)	(19)
B	H	J	L	R

[a]AFR = Africanized; EUR = European.
[b]Naturally mated.
[c]Single-drone, artificial insemination.

TABLE 3-5. Maternal effect: hypotheses and analyses (Mann-Whitney U test, one-tailed, alpha = 0.05). Letters refer to experimental treatments, see Table 3-4. The analyses of the following hypotheses (a postiori) demonstrate that the pupal weights of hybrids from a reciprocal F_1 cross are different from each other (H4) but are the same as their respective queen mothers (H2 and H6).

H1:	B < R	***[a]
H2:	B < H	NS
H3:	B < J	***
	B < L	***
H4:	H < J	***
	H < L	***
H5:	H < R	***
H6:	J < R	NS
	L < R	NS

[a] *** = P<0.001.

TABLE 3-6. Coefficients of variation for pupal weights from artificial, single drone inseminations and natural, multiple matings.

EGG GENOTYPE[a]	COMB CELL SIZE	
	AFRICANIZED	EUROPEAN
SINGLE DRONE INSEMINATIONS		
SDA12	3.9	3.6
SDY10	3.2	2.5
SDY11	2.8	3.5
SDY1	3.6	5.1
YD28	3.3	2.5
MULTIPLE INSEMINATIONS		
A26	4.4	4.5
A57	5.8	3.4
B39	3.1	5.1
WE1	3.4	2.0
ANALYSES[b]	NS	NS

[a]See Table 3-1 for explanation of genotypes.
[b]Mann-Whitney U test, one-tailed, alpha = 0.05.

CHAPTER IV
QUEEN DEVELOPMENT AND MATURATION

Introduction

African honey bees, Apis mellifera scutellata (formerly classified as adansonii; Ruttner 1976a, 1976b, 1981), were introduced into southeastern Brazil in 1956 (Kerr 1967; Michener 1975; Woyke 1969). The following year, swarms escaped and hybridized with the established European honey bees (primarily A. m. ligustica and mellifera) that had been introduced by 1845 (Gerstaker cited in Pellet 1938; Woyke 1969). The descendents from this hybridization are known as Africanized honey bees (Goncalves 1982).

Africanized honey bees in South America have a very high annual reproductive rate compared with European honey bees in temperate regions. Based on demographic data collected in French Guiana, the net reproductive rate for Africanized bees is estimated to be 16 colonies per colony per year (Otis 1980, 1982a). In comparison, the annual rate determined for European honey bees in North America was 0.92-0.96 (Seeley 1978) or 3-3.6 when afterswarms are considered (Winston 1980a; Winston, Taylor and Otis 1983). This dramatic difference in reproduction between these two honey bee populations may be a result of length of time throughout the year that resources are available in the tropics compared with temperate regions (see Chapter VIII) and/or

demographic characteristics of Africanized honey bees that account for high reproductive rates.

The reproductive rate of Africanized honey bees results in a swarm-to-swarm interval of approximately 90 days (Winston 1979b). During that period, a virgin queen emerges, develops pheromones necessary to attract drones, and mates; ovarian follicles mature; oviposition is initiated; and the colony population growth period begins prior to the next swarming. One expected demographic feature for a population with a high reproductive rate would be a short queen maturation interval (Fletcher 1977a). For the Africanized queens in French Guiana, the maturation interval from pupal eclosion to initiation of oviposition was 9.7 days (Otis 1980), over 10% of their swarm-to-swarm interval (calculated from Winston 1979b). Fletcher and Tribe (1977b) report that in the parental African population, oviposition begins on the 8th to 9th day after queen emergence. European queens begin ovipositing between the 6th and 17th day after emergence (Laidlaw and Eckert 1962; Oertel 1940; Root 1947). Otis (1980) calculated that the mean interval from pupal eclosion to oviposition for European queens (10.7 days) was not significantly different from that of Africanized queens (9.7 days). However, comparisons between reported values for both Africanized and European honey bees are inappropriate because the data were collected under very different experimental conditions. Therefore, this study was undertaken to determine if the queen maturation interval for Africanized honey bees is significantly different than that for European honey bees under identical conditions. Three aspects of queen maturation were evaluated: 1) larval, pupal and total development time from egg to adult emergence;

2) post-emergence development of queen attractiveness to drones; and 3) time from adult emergence to initiation of oviposition.

In the studies reported here, queen development and maturation were evaluated under controlled conditions. Total development time is defined as the time from oviposition to adult emergence. These experimental conditions avoid the problems of previous studies that evaluated queen development and maturation in colonies that were swarming (e.g., Otis 1980). Under natural swarming conditions, queens are very often confined within their cells by worker bees and prevented from emerging for 1-10 days after pupal eclosion (Otis 1980). Confinement makes calculations of development times difficult, and, because maturation proceeds during confinement, maturation time calculated from emergence to beginning of oviposition would be under estimated.

Methods

Queen Development Times

The Africanized egg source (A26) and the Africanized cell-producing colonies (A37 and A43) were established from queens removed from feral colonies found in an area of eastern Venezuela where there were no known European honey bees. They were identified as Africanized honey bees by their behavior and characteristic comb cell size (4.5-5.0 mm wide between opposite sides of the hexagon, see Chapter III). The European egg source (Y5) and the European cell-producing colonies (19, 27 28, F, H and H1) came from European queens commercially produced in the southeastern U.S.A. and shipped to Venezuela. European colony IBR was a stock supplied by the U.S. Department of Agriculture Bee Breeding and Stock Center Laboratory, Baton Rouge, Louisiana, USA.

Eggs of known ages were collected from the Africanized (A26) and European (Y5) egg sources by the standard commercial queen-producing technique of caging the queen on an empty comb within a colony (Harp 1973; Laidlaw 1979; see also Chapter II). After 6 hours, the combs with the egg samples were moved to strong incubator-colonies for the eggs to develop and larvae to hatch and be fed. Very young larvae, 12-18 hours old, were transferred (grafted) into beeswax queen-cell cups primed with royal jelly and then introduced into queen-cell-producing colonies (=nurse bee colonies) (Laidlaw 1979). All cell-producing colonies had large worker bee populations and were intentionally crowded into two standard Langstroth hive bodies. Queens in the cell-producing colonies were removed 48 hours before introducing the grafted cells. All young, unsealed brood was also removed 2-4 hours before introducing the grafted cells. Twenty grafted Africanized and twenty grafted European cells were introduced into each cell-producing colony. There were twenty cell cups to a frame, ten on the top bar and ten on the middle bar. Both Africanized and European larvae were grafted into the same frame, five each on the top bar and five each on the middle bar. All cell cups were equally spaced about 8 mm apart, centered on the bars.

In one experimental trial, the effect of nurse bee genotypes on queen development was evaluated by comparing queen development times for both Africanized and European egg genotypes in both Africanized and European cell-producing colonies. In another trial, development times for Africanized queens in Africanized and European cell-producing colonies were compared. In both trials, the Africanized cell-producing colonies had comb cell sizes characteristic of Africanized honey bees (4.8 mm wide; Chapter III).

The queen-cell-producing colonies were inspected only after the queen cells had been sealed in order to avoid disturbance which could affect development times. Once the cells are sealed, cell-producing colonies only maintain the appropriate temperature for the pupae to develop normally. On the sixth day after grafting, each sealed cell was protected by placing a 3 mm wire mesh tube around it to avoid any problems associated with queens being confined to their cells by worker bees. In addition, this also prevented any emerged virgin queens from destroying sealed cells that had not yet emerged. Beginning 24 hours before any expected queen emergence, the cell-producing colonies were inspected daily at 0630, 1200 and 1730 hours to record queen emergence. In two trials, cell-producing colonies were inspected daily at 0630, 1200 and 1730 hours, beginning 24 hours prior to estimated sealing time, in order to determine unsealed development times.

Development of Attractiveness of Virgin Africanized and European Honey Bee Queens to Drones

Two Africanized queen mothers (A26 and A57) were removed from feral colonies in eastern Venezuela. The two European queen mothers (We and Yk) were shipped to Venezuela from different commercial queen breeders in southeastern USA.

Queens from the four queen mothers (A26, A57, We and Yk) were produced as described above. Sealed queen cells were removed from the cell-producing colony and placed in an incubator (35 $\pm$ 1^{o}C) 48 hours prior to emergence. After emergence, the queens were marked for individual identification and maintained in separate cages in a queen storage colony (Laidlaw 1979).

In order to test for the degree of attractiveness to drones, each queen was tethered in a clean, plastic screen bag. The mesh size was 1

x 1 mm, and each bag was approximately 5 x 10 cm. Bags were individually suspended on monofilament line about 6.5 meters above the ground, centered between two poles 20 meters apart. Queens could be rapidly raised and lowered by a pulley system. Queens were put into the mesh bags just prior to testing in order to avoid any pheromone accumulation.

The testing location was in an open field in a drone congregation area (Zmarlicki and Morse 1963), which was located by walking with a helium-filled weather balloon with mature queens suspended 10-20 meters above the ground. The drone congregation area was identified when hundreds of drones oriented to the tethered queens. Boundaries appeared to be quite distinct and stable through time. Both Africanized and European drones were probably present, but the identity of each drone responding to specific queens during the experiment was not known because there are no reliable techniques to identify individual Africanized and European honey bees. How drone congregation areas become established is not understood, but these areas are probably where most mating occurs.

Individual queens were tested for drone response on consecutive days, beginning on the day of emergence. Only one queen at a time was tested so that the relative attraction of each queen would not be influenced by other queens being tested simultaneously. Testing lasted for a maximum of 3 minutes for each queen, even if no drone response was observed. Periodically, empty bags (blanks) were tested to insure that drones were responding only to the queens and not orienting to the experimental set-up and responding to the mesh bags. At no time did drones respond to the blanks. A random sequence for testing individual

queens was established on each day of the experiment. Each queen was tested more than once on each day and always in a new mesh bag, to avoid any pheromone accumulation or contamination. Each testing session was begun by suspending an older queen that had previously been determined to be maximally attractive, in order to insure that a responding drone population was available. This process was also repeated if the testing session was interrupted by rain, extreme cloudiness, or high winds--conditions that normally reduce drone flight activity. The testing took place between 1400 and 1600 hours.

Drone response was evaluated by assigning one of the following ranks to the test queen:

Rank 0 = no response

Rank 1 = drones oriented to the test subject but only flew past; no circling of the test subject

Rank 2 = drones oriented to the test subject and persisted in a wide circling formation more than 2 m from the subject

Rank 3 = drones oriented to the test subject and formed a loose comet-like formation down wind more than 0.5 m to the test subject; formation was volatile, continually fragmenting and reforming; drones did not land on the mesh bag

Rank 4 = drones oriented to the test subject and formed a tight comet-like formation down wind less than 0.5 m from the test subject; formation was persistent and did not fragment even as the test subject was lowered; drones landed on and walked over the mesh bag.

These ranking categories were easily discriminated and were not affected by the absolute numbers of drones flying. No estimates of the drone population were made.

Time Post-Emergence to the Initiation of Oviposition

Queens were produced as described above from one Africanized egg source (A26) and one European egg source (We). Twenty-seven Africanized and twenty-five European mature queen cells (two days prior to emergence) were each introduced into a four-frame queenless mating colony. Any natural queen cells in the mating colonies were destroyed before introducing the experimental queen cells. This insured that the only queen in the mating colony would be the experimental queen. When only one queen cell is present, worker bees usually do not confine her to her cell and the problem of calculating maturation time is avoided.

Because Africanized and European queens did not develop at the same rate, the day of queen emergence was determined by the mean time of emergence for a sample of sister queens from the same graft that were left to emerge in an incubator at 35 $\pm$ 1^{o}C. On the eleventh day after the queens emerged, the colonies were inspected and the age of the brood was evaluated to determine the age post-emergence when the queens had begun ovipositing. Those colonies in which there were no larvae were inspected three and five days later.

This experiment took place during the dry season. Clear weather prevailed so that mating flights were not affected by weather conditions. Both Africanized and European drones were in the area.

Results

Queen Development Times

Table 4-1 presents the experimental matrix for evaluating the interaction of egg genotype and nurse bee genotype on queen development times for Africanized and European queens. Table 4-2 presents the total development times from oviposition to adult emergence for Africanized queens and European queens in Africanized and European cell-producing colonies. Table 4-3 presents the analyses for the paired comparisons in each cell-producing colony. These paired comparisons avoid any differences between cell-producing colonies because colony size (nurse bee population), brood area temperature, and quantity and quality of larval food are factors that affect queen development (Beetsma 1979; Laidlaw 1979; Johansson and Johansson 1973). Africanized queens develop in 14.5 days post-oviposition compared with 15.0 days for European queens ($P<0.001$, Kolmogorov-Smirnov one-tailed test, chi-square distribution, $df = 2$; Siegel 1956). There was no significant effect of the cell-producing colony on queen development times (Kolmogorov-Smirnov two-tailed test, chi-square distribution, $df = 2$, alpha = 0.05) (Tables 4-4 and 4-5).

Table 4-6 presents the development times for the Africanized queens in Africanized and European cell-producing colonies. There was no difference in Africanized queen development time between Africanized and European cell-producing colonies (Kolmogorov-Smirnov one-tailed test, chi-square distribution, $df = 2$, alpha = 0.05).

The median unsealed development times from oviposition to sealing for both the Africanized and European queens was 7.5 days (Table 4-7). However, the Africanized and European genotypes were significantly

different as a result of the distribution around the median ($P<0.05$, Kolmogorov-Smirnov one-tailed test, chi-square distribution, df = 2).

Development of Attractiveness to Drones

The response of drones to tethered, virgin queens is summarized in Table 4-8. There were no differences between Africanized and European queens with respect to either the earliest age at which a positive drone response (Rank 1) was observed or the earliest age at which a maximum drone response (Rank 4) was observed. Both Africanized and European virgin queens were able to attract drones (Rank 1) on the day they emerged. Africanized virgin queens can maximally attract drones (Rank 4) by the fourth day post-emergence; European virgin queens can elicit a Rank 4 response by the fifth day post-emergence. This difference was not significant (Kolmogorov-Smirnov one-tailed test, chi-square distribution, df = 2, alpha = 0.05). The data in Table 4-8 have been combined for the two Africanized and two European queen lines. However, the queens within a population (Africanized or European) or within a line within a population were not uniform with respect to drone response or the rate of maturation. There were differences between the two Africanized lines and between individuals within the same line for the earliest age for a Rank 4 response. This same variation between lines and within lines existed for the European population.

Time Post-Emergence to Initiation of Oviposition

Table 4-9 presents the data for time post-emergence to the initiation of oviposition for both Africanized and European queens. Africanized queens began oviposition at 8.5 days post-emergence whereas European queens begin at 7.5 days ($P<0.05$, Kolmogorov-Smirnov two-tailed test, chi-square distribution, df = 2).

Discussion

Queen Development

Development time from oviposition to emergence for Africanized queens in this study was 14.5 days, which is the same as the development period reported for both Africanized bees in French Guiana (Winston 1979c) and their parental population, A. m. adansonii (=scutellata) in South Africa (Anderson, Buys and Johannsmeier no date; Fletcher 1978; Fletcher and Tribe 1977c). The European queens in the present study developed in 15.0 days, which is about one day shorter than expected from previous reports (Jay 1963; Laidlaw 1979). Therefore, the difference in development times for Africanized and European queens was not as great as expected and underscores the importance of making comparisons under the same experimental conditions.

Queens have approximately a 25% shorter development period than worker bees. Differences in total development times between queens and worker bees are primarily due to a much shorter sealed development stage, i.e., 7.5 days compared with 12 days for European bees and 7.0 days compared with 12 days for Africanized bees. The sealed development stage in worker bees is approximately 60% of the total development time, whereas in queens it is approximately 50%.

European queens took 3.4-5.6% longer than Africanized queens to develop. This difference is similar to the 5.3% difference in development times between European and Africanized worker bees from the same two egg sources (A26 and Y5) (see Chapter II).

There was no effect of nurse bee genotype on queen development times. However, queens were produced more successfully in European colonies. Africanized nurse bees were easily disturbed when the grafted

cells were introduced into the colonies, resulting in poor survival or acceptance of the grafted larvae (5-50% for Africanized colonies, compared with 35-95% for European colonies). In addition, Africanized colonies were difficult to manage because of excessive stinging that occurred when manipulating the strong colonies that were necessary for proper queen production.

Page and Erickson (1984) found evidence that nurse bee colonies preferentially raised queens from more closely related larvae. However, in the present study, no evidence for kin recognition was observed. Africanized and European nurse bee colonies reared Africanized and European queens with equal frequency (Table 4-2).

Rate of Maturation

Attractiveness of queens to drones is a function of the amount of pheromone (9-oxodec-trans-2-enoic acid) produced in the mandibular glands of the queens (Butler 1971; Boch, Shearer and Young 1975). In England, using European genotypes, Butler (1971) tethered virgin queens of various ages 6 meters above the ground in areas where drones were flying. He determined that queens younger than 5 to 6 days old seldom elicited a positive drone response. Maximum positive responses from drones were observed in queens 8 or more days old. Butler's results differ from those presented in this study and may be attributed to either differences in experimental conditions or genetic differences between the queen lines studied rather than to differences due to any tropical or temperate conditions. The response of drones to queens reported in this chapter was evaluated in a drone congregation area which may account for the differences between the studies.

In addition, Africanized drones (not present in Butler's study) may have a lower response threshold to queen pheromone and therefore would respond to queens with less pheromone present than would European drones. This hypothesis is suggested by the observation that there are differences in the sensory receptors on the antennae of Africanized drones compared with European drones (Dietz 1978). Further comparison between Africanized and European drones is needed to determine any differences in the threshold of response and whether or not this would give the Africanized drones a mating advantage.

Another factor that needs to be considered when using this behavioral bioassay (drone response) to compare rate of maturation of queens is that the pheromone is not continually produced but rather is pulsed in its production (R. Boch, pers. comm.). This factor may help to explain some of the variation of responses produced by queens within the same line. For example, in a few trials, a queen elicited a decreased response compared with the previous response she had elicited.

In the present study, the time post-emergence to the initiation of oviposition for Africanized queens was 8.5 days and for European queens was 7.5 days. There are no other data available that allow for valid comparisons. For example, Otis (1980) reports that the mean interval from emergence to initiation of oviposition was 7.8 days for Africanized queens in French Guiana. However, these data were collected by observing queen maturation in colonies that had swarmed and, therefore, the time from emergence to oviposition would be shortened because of a variable period of queen confinement [1-10 days (Otis 1980)] within the cells. In another set of data, Otis (1980) reports the mean maturation interval from eclosion to oviposition was 9.7 days. However, he does

not indicate how he determined when eclosion occurred, or if he was using the terms eclosion and emergence interchangeably. The normal time from eclosion to emergence for queens is approximately 12 hours (Jay 1963).

Because of their high reproductive rate and resultant short swarm-to-swarm interval, Africanized honey bees were expected to have a rapid queen maturation interval compared with European honey bees. Fletcher and Tribe suggest "that in the adansonii [=scutellata] race, natural selection has worked strongly in favour of minimizing the period between the loss of a queen [from swarming] and the re-establishment of oviposition by a new queen" (1977b, p. 167). The surprising result from this study was that both Africanized and European queens matured at approximately the same rate, determined both by their attractiveness to drones and the time from adult emergence to initiation of oviposition.

As Fletcher and Tribe (1977b) suggested, one would expect natural selection to be operating to minimize the maturation interval for queens, in order to maximize brood production between swarming periods. However, Africanized queens may be under a second and possibly more important selection pressure which may affect their maturation interval. Africanized swarms may travel great distances (Fletcher 1978; Michener 1975). Otis (1980) confirmed that at least some queens issuing with afterswarms had already mated. If new queens issuing with these swarms have mated prior to swarming or mate while enroute, then delayed maturation, particularly with respect to development of ovarian follicles, would be advantageous. Follicular development would increase the queen's weight and make it more difficult for her to fly. Prior to issuing with the prime swarm, older queens usually stop egg laying

several days before the swarm departs, allowing time for their ovaries to recess. Therefore, maturation for Africanized queens may be delayed in order for the swarms with new queens to be able to migrate long distances. Rather than selection operating to shorten the maturation interval, selection may be operating to delay maturation to enable long swarm migration distances.

The variation in queen maturation rates (see Tables 4-8 and 4-9) observed both within a population and within a queen line suggests that the physiological parameters involved in the process of maturation may be genetically determined. The rate of maturation is an important economic characteristic for commercial queen producers to consider in their selection programs. Reducing the time from emergence to initiation of oviposition can significantly increase the number of queens produced in each mating colony during the queen-producing season.

TABLE 4-1. Experimental matrix for the comparison of total development times (oviposition to adult emergence) for both Africanized and European honey bee queens.

	EGG GENOTYPES	
NURSE BEE GENOTYPE[a]	AFRICANIZED (A26)	EUROPEAN (Y5)
AFRICANIZED		
A43	A	B
A37	C	D
COMBINED	E	F
EUROPEAN		
19	G	H
27	I	J
28	K	L
F	M	N
H	O	P
IBR	Q	R
COMBINED	S	T
COMBINED AFRICANIZED AND EUROPEAN	U	V

[a]Queen-cell-producing colony.

TABLE 4-2. Total development times (in days from oviposition to adult emergence) for Africanized and European honey bee queens: median, (sample size).

NURSE BEE GENOTYPE[a]	EGG GENOTYPES	
	AFRICANIZED (A26)	EUROPEAN (Y5)
AFRICANIZED		
A43	14.0 (1)	15.0 (4)
A37	14.5 (7)	15.0 (4)
COMBINED	14.5 (8)	15.0 (8)
EUROPEAN		
19	14.5 (15)	15.0 (19)
27	14.5 (17)	15.0 (13)
28	14.0 (13)	14.5 (12)
F	14.0 (10)	14.5 (9)
H	14.0 (7)	14.5 (8)
IBR	14.0 (8)	14.8 (8)
COMBINED	14.2 (70)	15.0 (69)
COMBINED AFRICANIZED AND EUROPEAN	14.5 (78)	15.0 (77)

[a]Queen-cell-producing colony.

TABLE 4-3. Analyses for the comparison of queen development times for both Africanized and European honey bee genotypes. Letters A - V represent different treatments; see Table 4-1 for explanation. Kolmogorov-Smirnov one-tailed test, chi-square distribution, df = 2, alpha = 0.05 (Siegel 1956).

A x B	NS[a]
C x D	NS[a]
E x F	*[b]
G x H	***
I x J	**
K x L	**
M x N	**
O x P	*
Q x R	*
S x T	***
U x V	***

[a]Small sample size, chi-square distribution is conservative.
[b] * = P<0.05
** = P<0.01
*** = P<0.001.

TABLE 4-4. Analyses of queen development times for the Africanized egg genotype in the different cell-producing colonies. Letters represent different cell-producing colonies; see Table 4-1 for explanation. Kolmogorov-Smirnov two-tailed test, chi-square distribution, df = 2, alpha = 0.05 (Siegel 1956).

A x C	NS
A x G	NS
A x I	NS
A x K	NS
A x M	NS
A x O	NS
A x Q	NS
C x G	NS
C x I	NS
C x K	NS
C x M	NS
C x O	NS
C x Q	NS
G x I	NS
G x K	NS
G x M	NS
G x O	NS
G x Q	NS
I x K	NS
I x M	NS
I x O	NS
I x Q	NS
K x M	NS
K x O	NS
K x Q	NS
M x O	NS
M x Q	NS
O x Q	NS

TABLE 4-5. Analyses of queen development times for the European egg genotype in the different cell-producing colonies. Letters represent different cell-producing colonies; see Table 4-1 for explanation. Kolmogorov-Smirnov two-tailed test, chi-square distribution, df = 2, alpha = 0.05 (Siegel 1956).

B x D	NS
B x H	NS
B x J	NS
B x L	NS
B x N	NS
B x P	NS
B x R	NS
D x H	NS
D x J	NS
D x L	NS
D x N	NS
D x P	NS
D x R	NS
H x J	NS
H x L	NS
H x N	NS
H x P	NS
H x R	NS
J x L	NS
J x N	NS
J x P	NS
J x R	NS
L x N	NS
L x P	NS
L x R	NS
N x P	NS
N x R	NS
P x R	NS

TABLE 4-6. Total development time (in days from oviposition to adult emergence) of Africanized queens in Africanized and European cell-producing colonies: median, (sample size).

NURSE BEE GENOTYPE[a]	AFRICANIZED EGG GENOTYPE (A26)	
AFRICANIZED[b]		
A43	14.4 (10)	A
A37	14.6 (6)	B
A43 & A37	14.4 (16)	C
EUROPEAN		
H1	14.4 (16)	D
IBR	14.2 (14)	E
H1 & IBR	14.4 (30)	F
ANALYSES[c]		
A x D	NS	
A x E	NS	
B x D	NS	
B x E	NS	
C x F	NS	

[a]Queen-cell-producing colonies.
[b]Africanized comb cell size.
[c]Kolmogorov-Smirnov one-tailed test, chi-square distribution, df = 2, alpha = 0.05.

TABLE 4-7. Unsealed (egg and larval periods combined) development times (in days) for Africanized and European queens: median, (sample size).

NURSE BEE GENOTYPE[a]	EGG GENOTYPES: AFRICANIZED (A26)	EGG GENOTYPES: EUROPEAN (Y5)	EFFECT OF EGG GENOTYPE[b]
19	7.5 (17)	7.5 (19)	*
28	7.2 (14)	7.5 (12)	*
19 & 28	7.5 (31)	7.5 (31)	*
EFFECT OF NURSE BEE GENOTYPE[c]	NS	NS	

[a]Cell-producing colonies, European nurse bees, European comb cell size.
[b]Kolmogorov-Smirnov one-tailed test, chi-square distribution, df = 2, * = $P<0.05$.
[c]Kolmogorov-Smirnov two-tailed test, chi-square distribution, df = 2, alpha = 0.05.

TABLE 4-8. Drone response to tethered virgin queens: median day of response post-emergence, range, (sample size).

	DAY OF RESPONSE LEVEL			
	RANK 1	RANK 2	RANK 3	RANK 4
AFRICANIZED QUEEN GENOTYPES	0[a] -- (2)	3.5 1-5 (6)	3.5 1-5 (6)	4.0 1-5 (5)
EUROPEAN QUEEN GENOTYPES	0[a] -- (1)	1.5 1-5 (4)	4.0 2-5 (3)	4.5 4-5 (2)
ANALYSES[b]	--	NS	NS	NS

[a]Day 0 = day of adult emergence.
[b]Kolmogorov-Smirnov one-tailed test, chi-square distribution, df = 2, alpha = 0.05.

TABLE 4-9. Time post-emergence to initiation of oviposition: median, range, (sample size).

	DAYS POST-EMERGENCE
AFRICANIZED GENOTYPE (A26)	8.5 7.5-12 (10)
EUROPEAN GENOTYPE (We)	7.5 6-10 (16)
ANALYSIS[a]	P<0.05

[a]Kolmogorov-Smirnov two-tailed test, chi-square distribution, df = 2.

CHAPTER V
QUEEN PUPAL WEIGHTS

Introduction

Africanized honey bees in South America are hybridized descendents of African honey bees (Apis mellifera scutellata) and European honey bees (primarily A. m. ligustica and A. m. mellifera) (Goncalves 1982; Woyke 1969). The annual net reproductive rate of Africanized honey bees in South America is four to five times greater than that of European honey bees in temperate regions: 16 colonies per colony per year compared with 3-3.6 (Otis 1980, 1982a; Winston 1980a; Winston, Taylor and Otis 1983). Differences in reproductive rates between these two honey bee populations may be a result of: 1) colony demography; 2) temperate vs. tropical climate and floral resources; 3) resource utilization behaviors; or 4) a combination of factors. Because Africanized and European honey bees have not been compared under identical experimental conditions, it is not possible to determine to what extent reproductive differences are a result of genetic or environmental parameters.

One demographic parameter associated with rapid colony growth and a high rate of colony reproduction would be a high oviposition rate (Brian 1965; Moeller 1961; Wilson 1971). In the evolution of social insects, queen oviposition rates have increased primarily due to one of the following: increased number of ovarioles, increased length of the

ovarioles, more rapid egg maturation, and reduction in egg size (Hagan 1954 and Iwata and Sakagami 1966 cited in Wilson 1971; Wilson 1971). Honey bee queens have a very large number of ovarioles (>300) and, for European queens, the number of ovarioles has been shown to be an inherited character (Eckert 1934) which is positively correlated with queen pupal weight (Hoopingarner and Farrar 1959). Queen weight was also found to be correlated with brood production (Boch and Jamieson 1960). If it is assumed that both Africanized and European honey bees have the same relationship between queen weight and brood production, then weights of queens from the two populations can be compared to determine potential differences in fecundity.

In honey bees, differentiation between worker and queen castes is not genetically determined, but rather is regulated by the quantity and quality of food fed to developing larvae during the first 3 days (Beetsma 1979). Therefore, a number of factors other than genotype affect queen size, e.g., age of larvae used to produce queens, population of the cell-producing colony, quantity and quality of food fed to developing larvae, and temperature (Beetsma 1979; Johansson and Johansson 1973; Laidlaw 1979; Weiss 1974; Woyke 1971). Because of differences in queen rearing methods and experimental conditions, previous comparisons of size between Africanized and European queens may be inappropriate. This study was undertaken to compare queen pupal weights for Africanized and European honey bees under identical experimental conditions in Venezuela.

Methods

Four Africanized honey bee lines (A26, A57, A61 and A62) were established from queens removed from feral colonies in an area in

eastern Venezuela that had no known European honey bees. They were identified as Africanized honey bees by their comb cell size, which was significantly smaller than European comb cell size (Chapter III). Two European lines (YK and WE) were established from queens shipped to Venezuela by commericial queen producers in the southeastern U.S.A. Three additional European lines (YD, N and GK) were established from queens shipped to Venezuela from the U.S. Department of Agriculture Bee Breeding and Stock Center Laboratory, Baton Rouge, Louisiana, U.S.A.

Queens were produced from these nine lines by standard queen rearing methods (Laidlaw 1979). Egg samples from the nine queen mothers were collected by confining the queens to an empty comb within their own colonies using an 8 x 8 cm push-in cage made from 3 mm mesh hardware cloth. Queen excluder material was soldered to the tops of the push-in cages, allowing worker bees to move in and out in order to feed and tend the queen (Harbo, Bolten, Rinderer and Collins 1981). Both Africanized and European eggs were collected in European size comb. After approximately 4-6 hours, the queens were released, and combs containing the eggs were put into a strong colony in order for the eggs to be incubated and for the larvae to be fed. Africanized and European eggs were both put into the same incubator-colony in order to control for any differences in early larval feeding and temperature.

Young larvae approximately 12-15 hours old were transferred (grafted) into artificial, beeswax, queen-cell cups and then introduced into the cell-producing colonies. Twenty larvae from one of the Africanized lines and twenty larvae from one of the European lines were grafted into each cell-producing colony. To control for extrinsic factors affecting queen size, analyses of Africanized and European

queens were limited to paired comparisons (one Africanized and one European line) that were each simultaneously introduced into the same incubator-colony and then grafted into the same queen-cell-producing colony. Possible effects from different cell-producing colonies on queen pupal weight were evaluated by grafting the same queen lines into different cell-producing colonies.

Only European cell-producing colonies were used because of the difficulty in producing queens in Africanized colonies. Africanized cell-producing colonies remained disturbed for a long period of time after the grafted larvae were introduced, which resulted in poor acceptance (survival) of the larvae (see Chapter IV).

Queen pupal weights are used for comparison because adult weights vary with respect to engorgment of food, dehydration, feces accumulation, and differential ovariole development. Although queen pupal weights vary with age of the pupae, there is a period from the 10th through the 13th day post-oviposition when queen pupal weight is constant (Table C-1). Queen pupal weight comparisons can therefore be made during this period (Hoopingarner and Farrar 1959). Although there is a 0.5 day difference in development time between Africanized and European queens (Chapter IV), the 3-4 day pupal period during which there is no significant weight change is of sufficient duration to allow Africanized and European queens to be accurately and consistently compared. Africanized and European queen pupae were weighed on the 11th day post-oviposition. Queen cells from each of the lines were randomly selected to avoid any position effect from location on the grafting frame. Weights were measured to the nearest 1.0 mg using either a Mettler Type H4 or H6 balance.

Queen cell lengths were measured at the time the queen pupal weights were determined. A calipers was used to determine the external length from the base to the apex of the queen cell.

Results

Queen pupal weights for four Africanized and five European lines are presented in Table 5-1. European queen pupal weights were significantly larger than Africanized queen pupal weights for two different pairwise comparisons, (YK vs. A26 and YK vs. A57; $P<0.05$ to $P<0.001$; Mann-Whitney U test, one-tailed). Africanized queen pupal weights were significantly larger in one pairwise comparison (A62 vs. N; $P<0.02$; Mann-Whitney U test, two-tailed). For three pairwise comparisons, there was no statistical difference (A26 vs. WE, A57 vs. YD, and A61 vs. GK; Mann-Whitney U test, one-tailed, alpha = 0.05). Because different cell-producing colonies had no significant effect on queen pupal weights (see below), the means for the nine queen lines can be ranked and analyzed (Table 5-2). There was no significant difference between the Africanized and European honey bee populations for queen pupal size (Mann-Whitney U test, one-tailed, alpha = 0.05).

Queen cell lengths for Africanized and European lines are presented in Table 5-3. In six out of eight pairwise comparisons, there was no significant difference in queen cell lengths between Africanized and European queens (Mann-Whitney U test, one-tailed, alpha = 0.05). For the pair in cell-producing colony 2, the European line was significantly larger than the Africanized line ($P<0.05$). For the pair in cell-producing colony 4, the Africanized line was significantly larger than the European line ($P<0.02$; Mann-Whitney U test, two-tailed).

Spearman's rank correlation coefficient was determined for queen pupal weights and queen cell lengths (Table 5-4). In general, there was no significant correlation between queen pupal weight and queen cell length (alpha = 0.05). However, one Africanized line (A26) in cell-producing colony 2 had a significant correlation ($P<0.05$) and one European line (YK) in cell-producing colony 4 had a significant correlation ($P<0.01$).

The effect of cell-producing colonies on queen pupal weights and queen cell lengths is presented in Table 5-5. There was no significant difference for Africanized queen line A26 in four different cell-producing colonies (one-way analysis of variance, alpha = 0.05); nor was there a significant difference for Africanized queen line A57 in two different cell-producing colonies (Mann-Whitney U test, two-tailed, alpha = 0.05). There was no significant difference in pupal weights for the European queen line YK in four different cell-producing colonies (one-way analysis of variance, alpha = 0.05), but there was a significant effect of cell-producing colonies on queen cell length ($P<0.001$). When cell-producing colony 2 was removed from the analysis, there was no significant difference in queen cell length.

Discussion

If we assume for both Africanized and European honey bees that queen weight is correlated with egg production or fecundity (Boch and Jamieson 1960), we would then expect that egg laying rates would follow the same ranking as presented in Table 5-2 for queen pupal weights. Based on these pupal weights, we would predict that there would be no difference in egg laying rates for the Africanized and European honey bee populations. In fact, when egg laying rates for Africanized and

European honey bee queens were compared, there was no significant difference between queens from the two populations (Chapter VI). There were, however, significant differences in pupal weights between individual queen lines both between and within each population (Table 5-1). There were also significant differences in egg laying rates between individual queen lines both between and within the two populations (Chapter VI).

In this study, Africanized queens were reared in European colonies because of low acceptance (survival) of grafted cells in Africanized colonies (Chapter IV). Because queen-worker caste differentiation in honey bees is regulated by larval feeding (Beetsma 1979), rearing Africanized queens in European colonies may have obscured differences in pupal weights between Africanized and European queens. Possibly, European worker bees may rear larger Africanized queens than would Africanized worker bees because European worker bees are themselves larger (Chapter III), and may feed the developing queen larvae differently. Although virgin European queens have been reported to weigh more than virgin Africanized queens--208 vs. 199 mg (Goncalves, Kerr and Nocoes 1972 cited in Michener 1975)--there was no indication of conditions under which the queens were reared.

Further analysis of queen weights between Africanized and European honey bees is needed, preferably in a 2 x 2 experimental design: Africanized and European queens reared in both Africanized and European cell-producing colonies. In addition, the relationship between queen pupal weights and brood production needs to be evaluated for both Africanized and European honey bee lines to determine if the same relationship exists for both populations.

The European queen lines evaluated were a diverse representation of the European population from North America, whereas the Africanized queen lines may only reflect a small portion of the Africanized population. The location for the sources of the Africanized lines was limited to feral colonies found in one area of eastern Venezuela. A greater diversity of Africanized lines needs to be evaluated in order to be able to generalize about queen pupal weights and oviposition rates for the population as a whole.

TABLE 5-1. Comparison of Africanized and European queen pupal weights (mg): mean ± SD, (sample size), (genotype).

CELL BUILDER[a]	AFRICANIZED GENOTYPES	EUROPEAN GENOTYPES	ANALYSES[b]
1	257.1 ± 7.6 (9) (A26)	286.4 ± 12.0 (9) (YK)	***
2	255.8 ± 9.2 (9) (A26)	284.4 ± 20.6 (9) (YK)	**
3	262.3 ± 6.4 (3) (A26)	266.7 ± 9.7 (9) (WE)	NS
4	260.0 ± 9.0 (11) (A57)	272.5 ± 16.5 (13) (YK)	*
5	243.0 ± 13.4 (3) (A26)	282.6 ± 12.0 (3) (YK)	*
6	248.6 ± 14.3 (4) (A57)	264.0 ± 15.4 (5) (YD)	NS
7	291.8 ± 7.9 (5) (A62)	257.3 ± 18.2 (4) (N)	--[c]
8	237.2 ± 21.7 (5) (A61)	232.9 ± 0.4 (2) (GK)	NS

[a]Cell-producing colonies; European nurse bees and European comb cell size.
[b]Mann-Whitney U test, one-tailed, alpha = 0.05;
* = P<0.05, ** = P<0.01, *** = P<0.001.
[c]Difference in wrong direction for one-tailed test; two-tailed test results in a P<0.02.

TABLE 5-2. Queen pupal weights for the nine lines analyzed.

POPULATION	QUEEN LINE	MEAN PUPAL WEIGHT(MG)
European	GK	233
Africanized	A61	237
Africanized	A26	256
European	N	257
Africanized	A57	257
European	YD	264
European	WE	267
European	YK	280
Africanized	A62	292
ANALYSIS[a]		NS

[a]Mann-Whitney U test, one-tailed, alpha = 0.05.

TABLE 5-3. Comparison of Africanized and European queen cell lengths (mm): mean ± SD, (sample size), (genotypes).

CELL BUILDER[a]	AFRICANIZED GENOTYPES	EUROPEAN GENOTYPES	ANALYSES[b]
1	2.58 ± 0.1 (9) (A26)	2.50 ± 0.1 (9) (YK)	NS
2	2.62 ± 0.2 (9) (A26)	2.70 ± 0.1 (9) (YK)	*
3	2.54 ± 0.1 (3) (A26)	2.48 ± 0.1 (9) (WE)	NS
4	2.58 ± 0.1 (7) (A57)	2.44 ± 0.1 (11) (YK)	--[c]
5	2.67 ± 0.1 (3) (A26)	2.57 ± 0.1 (3) (YK)	NS
6	2.50 ± 0.02 (4) (A57)	2.57 ± 0.1 (5) (YD)	NS
7	2.78 ± 0.04 (5) (A62)	2.81 ± 0.1 (3) (N)	NS
8	2.79 ± 0.1 (5) (A61)	2.76 ± 0.1 (2) (GK)	NS

[a]Cell-producing colonies; European nurse bees and European comb cell size.
[b]Mann-Whitney U test, one-tailed, alpha = 0.05; * = P<0.05.
[c]Difference in wrong direction for one-tailed test; two-tailed test results in a P<0.02.

TABLE 5-4. Correlation of queen cell length (mm) and queen pupal weight (mg): mean ± SD, (sample size). Measurement made on day 11.25 post-oviposition.

QUEEN GENOTYPE	QUEEN CELL LENGTH	QUEEN PUPAL WEIGHT	CORRELATIONS[a]
AFRICANIZED			
A26 (CB1)[b]	2.58 ± 0.1 (9)	257.1 ± 7.6 (9)	NS
A26 (CB2)	2.62 ± 0.2 (9)	255.8 ± 9.2 (9)	*
A26 (CB3)	2.54 ± 0.1 (3)	262.3 ± 6.4 (3)	--
A26 (CB5)	2.67 ± 0.1 (3)	243.0 ± 13.4 (3)	--
A57 (CB4)	2.58 ± 0.1 (7)	259.1 ± 10.8 (7)	NS
A57 (CB6)	2.50 ± 0.02 (4)	248.6 ± 14.3 (4)	NS
A62 (CB7)	2.78 ± 0.04 (5)	291.8 ± 7.8 (5)	NS
A61 (CB8)	2.79 ± 0.1 (5)	237.2 ± 21.7 (5)	NS
EUROPEAN			
YK (CB1)	2.50 ± 0.1 (9)	286.4 ± 12.0 (9)	NS
YK (CB2)	2.70 ± 0.1 (9)	284.4 ± 20.6 (9)	NS
YK (CB4)	2.44 ± 0.1 (11)	272.2 ± 17.8 (11)	**
YK (CB5)	2.57 ± 0.1 (3)	282.6 ± 12.0 (3)	--
WE (CB3)	2.48 ± 0.1 (8)	268.4 ± 8.8 (8)	NS
YD (CB6)	2.57 ± 0.1 (5)	264.0 ± 15.4 (5)	NS
N (CB7)	2.81 ± 0.1 (3)	264.3 ± 14.0 (3)	--
GK (CB8)	2.76 ± 0.1 (2)	232.9 ± 0.4 (2)	--

[a]Spearman's rank correlation coefficients, alpha = 0.05; * = $P<0.05$; ** = $P<0.01$.
[b]CB = cell-producing colony number; refer to Table 5-1 for explanation.

TABLE 5-5. Effect of cell-producing colony on queen cell length and queen pupal weight: mean ± SD, (sample size).

AFRICANIZED QUEEN GENOTYPE (A26)

	CB1[a]	CB2	CB3	CB5	ANALYSES[b]
QUEEN CELL LENGTH	2.58 ± 0.1 (9)	2.62 ± 0.2 (9)	2.54 ± 0.1 (3)	2.67 ± 0.1 (3)	NS
QUEEN PUPAL WEIGHT	257.1 ± 7.6 (9)	255.8 ± 9.2 (9)	262.3 ± 6.4 (3)	243.0 ± 13.4 (3)	NS

AFRICANIZED QUEEN GENOTYPE (A57)

	CB4	CB6	ANALYSES[c]
QUEEN CELL LENGTH	2.58 ± 0.1 (7)	2.50 ± 0.02 (4)	NS
QUEEN PUPAL WEIGHT	260.0 ± 9.0 (11)	248.6 ± 14.3 (4)	NS

EUROPEAN QUEEN GENOTYPE (YK)

	CB1	CB2	CB4	CB5	ANALYSES[b]
QUEEN CELL LENGTH	2.50 ± 0.1 (9)	2.70 ± 0.1 (9)	2.44 ± 0.1 (11)	2.57 ± 0.1 (3)	***[d]
QUEEN PUPAL WEIGHT	286.4 ± 12.0 (9)	284.4 ± 20.6 (9)	272.5 ± 16.5 (13)	282.6 ± 12.0 (3)	NS

[a]CB = cell-producing colony number; refer to Table 5-1 for explanation.
[b]One-way analysis of variance, alpha = 0.05; *** = P<0.001.
[c]Mann-Whitney U test, two-tailed, alpha = 0.05.
[d]With CB2 removed, ANOVA is NS.

CHAPTER VI
EGG-LAYING AND BROOD PRODUCTION RATES DURING THE FIRST BROOD CYCLE

Introduction

Africanized honey bees in South America are descendents from the hybridization of African honey bees (Apis mellifera scutellata) and European honey bees (primarily A. m. ligustica and A. m. mellifera) (Goncalves 1982; Woyke 1969). In tropical and sub-tropical regions of South America, Africanized bees have been more successful than European bees as determined by their rapid rates of dispersal and high population densities (Michener 1975; Taylor 1977, 1985). It is not surprising that Africanized bees are more successful in these regions because they are descendents of honey bees that evolved under similar tropical conditions in Africa.

Rates of dispersal and population densities achieved by Africanized honey bees require a high colony reproductive rate. Africanized honey bees in South America have a reproductive rate that is four to five times greater than the reproductive rate of European honey bees in North America (Otis 1980, 1982a; Winston 1980a; Winston, Taylor and Otis 1983). However, based on this comparison, one cannot identify the factors that account for differences in reproductive rates nor identify the factors leading to the success of Africanized bees in South America. Because the comparison of reproductive rates was not based on data collected under similar environmental or experimental conditions, it is

not possible to determine to what extent reproductive differences between the two populations are a result of differences in intrinsic demographic parameters and/or environmental differences due to temperate vs. tropical resources and climatic conditions. In addition, experimental conditions were very different. For example, an important variable affecting reproductive rates in honey bees is brood-nest crowding (Baird and Seeley 1983; Simpson 1966, 1973; Simpson and Riedel 1963). Experimental Africanized colonies in South America were maintained in 22-liter hives (Otis 1980; Winston 1979b), whereas, experimental European colonies in North America, with which they were compared, were maintained in 42-liter hives (Winston 1980a).

Reproductive rates in honey bees are a result of an interaction of at least three factors: colony demography, resource availability, and resource utilization efficiency. As part of a larger investigation comparing intrinsic demographic factors between Africanized and European honey bees to determine which aspects of demography, if any, are responsible for the success of Africanized honey bees in South America, this study evaluated one aspect of demography--queen fecundity. Queen egg laying rate is one of the primary demographic parameters that affects colony growth rates (Brian 1965; Moeller 1961; Wilson 1971). Although differences in egg laying rates between Africanized queens and European queens have been reported (Fletcher 1978; Michener 1972, 1975; Ribbands 1953), they cannot be compared because the data were collected under different resource and experimental conditions. Therefore, the present study was undertaken to compare egg laying and brood production rates for both Africanized and European queens under identical, tropical conditions in Venezuela. The experimental design allowed for a

comparison between the two honey bee populations during the first brood cycle. Differences in initial colony growth rates between Africanized and European honey bees may be an important factor in determining differences in reproductive rates.

Egg laying and brood production rates for Africanized and European honey bees were evaluated at both the queen and worker bee levels. The interactions of both Africanized and European queens with both Africanized and European worker bees were evaluated because of potential behavioral and/or physiological differences between Africanized and European nurse bees with respect to affecting brood production rates and/or the queen's oviposition behavior. In order to compare egg laying and brood production rates between Africanized and European honey bees, four variables needed to be controlled.

First is colony size, because egg laying rates are positively correlated with the number of worker bees in a colony (Moeller 1958). In order to evaluate initial colony growth, a colony size was selected that contained the number of worker bees within the range reported for both Africanized and European swarms (Fell et. al. 1977; Otis 1980; Rinderer, Collins, Bolten and Harbo 1981; Rinderer, Tucker and Collins 1982; Winston 1980a).

Second is hive cavity volume, which must be controlled in order to avoid effects of differential brood-nest crowding on oviposition rates (Brian 1965). A hive cavity volume was selected that represents natural nest cavity volumes chosen by these two honey bee populations (Rinderer, Collins, Bolten and Harbo 1981; Rinderer, Tucker and Collins 1982; Seeley 1977; Seeley and Morse 1976; Winston, Taylor and Otis 1983).

A third variable, comb cell size, had to be controlled. If bees were allowed to build their own comb, comb built by Africanized workers would be smaller than comb built by European bees (Chapter III). The larger, European comb was selected for these experiments for two reasons: 1) European queens do not lay eggs in a uniform pattern in Africanized comb; and 2) there is a higher brood mortality in colonies with European nurse bees on Africanized comb--possibly because the larger European nurse bees have difficulty feeding the developing larvae in the smaller, Africanized cells (Chapters II and III). On the other hand, Africanized queens and worker bees appear to behave normally when managed on European comb. Only Africanized bees that had been reared in managed colonies with European combs were used in the experimental colonies to avoid any delay in adjusting to larger comb cell size.

Finally, the fourth variable controlled was resource availability. Africanized and European queens were compared simultaneously so that floral resource conditions were identical. Surplus honey was also provided for each experimental colony to reduce the effects of differential foraging success between Africanized and European honey bees in tropical resource conditions (Rinderer, Bolten, Collins and Harbo 1984; Rinderer, Collins and Tucker 1985).

In addition to colony-level variables, conditions under which experimental queens are produced may affect queen fecundity, e.g., age of larvae used to produce queens, quantity and quality of food fed to developing queen larvae, and temperature during development (Beetsma 1979; Laidlaw 1979; Weiss 1974; Woyke 1971). The age of a queen may also affect her fecundity (Ribbands 1953). In this study, all

experimental queens were produced under identical conditions and were the same age.

Estimates of daily egg laying rates derived from total brood production may not be accurate (Merrill 1924) because mortality of unsealed brood (eggs and larvae) may be quite high, up to 50% (Garofalo 1977; Merrill 1924; Woyke 1977), particularly during the initial period of colony growth (Winston, Dropkin and Taylor 1981). Therefore, both daily egg laying rates and brood production during the first brood cycle were analyzed.

Methods

Daily Egg Laying Rates

Three different Africanized queen mothers (A57, A61 and A62) were established from feral colonies that were found in an area of eastern Venezuela with no known European honey bees. They were confirmed as Africanized honey bees by their behavior and comb cell size (Chapter III). Three different European queen mothers were shipped to Venezuela from the U.S.A.; two (GK and YD) were from the U.S. Department of Agriculture Bee Breeding and Stock Center Laboratory in Baton Rouge, Louisiana, and the third (YK) was from a commercial queen producer in southeastern U.S.A.

Experimental queens were produced from the queen mothers using standard queen rearing techniques (Laidlaw 1979). Eggs from the queen mothers were collected by restricting queens to a portion of comb for 4 to 6 hours under 8 x 8 cm push-in cages that had queen excluder material soldered to the tops (Harbo, Bolten, Rinderer and Collins 1981). Combs with the egg samples were then placed into a populous colony where the eggs were incubated until the larvae hatched and were fed. Young

larvae, approximately 15 hours old, were transferred (grafted) into beeswax queen-cell cups that had been primed with diluted royal jelly and then introduced into cell-producing colonies for development. Three days prior to adult emergence, sealed queen cells were put into an incubator (35 $\pm$ 1° C.). Emerged, virgin queens were marked for individual identification, using color-coded, plastic, numbered discs glued to the queen's thorax (Smith 1972). Virgin queens were maintained separately in small, two-frame colonies during the period of maturation and after artificial insemination in order to maximize the number of spermatozoa that migrate to the spermatheca (Woyke 1979).

Queens were artificially inseminated one week after emergence using the apparatus designed by Harbo (1979) and Mackensen (Mackensen and Roberts 1948; Mackensen and Tucker 1970). Queens were inseminated with 2.5 ul of wild-type semen on each of two occasions, 3 days apart, to increase the percentage of spermatozoa entering the spermatheca (Bolten and Harbo 1982; Mackensen 1964).

Four colony treatments were established:

1. Africanized queen with Africanized worker bees,
2. Africanized queen with European worker bees,
3. European queen with Africanized worker bees and
4. European queen with European worker bees.

Each experimental colony was in a five-frame hive (23 liters) that contained three empty combs of European cell size. A different geometric design was painted over the entrance of each hive in order to aid in orientation and reduce drifting of foragers between colonies (von Frisch 1967).

Two days prior to the beginning of the experiment, young bees were removed from brood frames of large colonies and put into screened cages that measured 48 x 37 x 76 cm. One cage contained Africanized bees and the other contained European bees. Africanized bees were removed only from colonies that were being managed on combs of European cell size. The cages were supplied with feeders containing 50% (volume:volume) sugar syrup. Bees used to stock the experimental colonies were taken from the appropriate cage, thereby insuring that all experimental colonies were uniform in composition for a particular worker bee type, Africanized or European. Each experimental colony was started with approximately 775 grams of bees.

Test queens were introduced into each experimental colony using a push-in cage (Laidlaw 1979). Queens were manually released from the push-in cages after two days.

The experiment consisted of two separate trials. Trial 1 evaluated colony treatments 2 and 4 only. Trial 2 evaluated all four colony treatments simultaneously. Trial 2 was started with a new supply of worker bees. Some of the queens used in trial 2 had also been evaluated in trial 1.

Egg laying rates were determined by removing the frames from each colony every 24 hours and counting the number of eggs. The removed frames were immediately replaced with empty frames in order to minimize disturbance. The frames with eggs were stored in a freezer until the eggs were counted. Initial egg laying rates that were more than two standard deviations from the mean were not used because these rates may have occurred before queen maturation was complete. Experimental colonies with Africanized worker bees (trial 2) began absconding

(abandoning the hive) after five to six days because of the disturbance caused by the experimental procedure. The experiment was terminated at that point and only egg laying rates prior to the beginning of absconding were compared.

Brood Production Rates during First Brood Cycle

The same queens evaluated in the egg laying experiment were used for this experiment. They were maintained in separate cages in a queen storage colony (Laidlaw 1979; Reid 1975) for six days between experiments. Four colony treatments were established as described above, except that the hives were larger and were stocked with more worker bees. Standard Langstroth hive bodies (48 liters) were used with nine frames (all with European comb cell size): eight empty, drawn combs and one filled with honey. All frames were weighed prior to being put into the colonies in order that weight changes could be monitored. Different geometric designs were placed above the entrances to facilitate orientation and reduce drift between colonies.

As described for the first experiment, young worker bees were collected and maintained in screened cages for two days prior to the beginning of the experiment. Approximately 1200 grams of worker bees were used to stock each colony. The number of bees put into each colony was estimated by determining the mean individual bee weight from three 20-30 bee samples for each cage and then dividing the total weight of introduced bees by the mean individual bee weight (Otis 1982b).

Queens were introduced into the experimental colonies using push-in cages and manually released two days later in order to standardize the starting day (day 0) for all experimental colonies. Queens that had been in Africanized colonies for the daily egg laying rate experiment

were now introduced into European colonies; queens that had been in European colonies during the first experiment were now introduced into Africanized colonies.

After day 12 of oviposition, each experimental colony was inspected and the amounts of unsealed brood (eggs and larvae) and sealed brood (pre-pupae and pupae) for each colony were determined. To facilitate measuring the amounts of unsealed and sealed brood, a 2.5 x 2.5 cm grid was placed over each frame and the amount of brood within each square was estimated. The number of developing brood cells was determined by multiplying the brood area (in cm^2) by 4.25 (the number of comb cells per cm^2). The number of worker bees present at day 12 was estimated by assuming a constant rate of mortality of adults from day 0 to day 17.

At the end of day 17 of oviposition, colonies were closed and killed with potassium cyanide. Unsealed and sealed brood, numbers of adult bees, amount of pollen, and frame weights were determined.

The number of adult bees present at day 17 was estimated by determining the mean individual bee weight from three samples of 150-200 bees taken from each colony. The total weight of bees in each colony was then divided by the mean individual weight to get an estimate of the total number of bees in each colony (Otis 1982b). The accuracy of this technique was determined by comparing the estimates with the actual counts (Table D-1). A mean difference of only 1.5% was observed.

Data were analyzed using the Mann-Whitney U test (alpha = 0.05). Correlations were evaluated using Spearman rank correlation coefficients (alpha = 0.05).

Results

Daily Egg Laying Rates

There were no significant differences in daily egg laying rates between Africanized and European queens (Tables 6-1 to 6-5). There were, however, significant differences between individual queen lines both between the two populations and within each population (Table 6-6).

There was no significant effect of worker bee type (Africanized vs. European) on egg laying rates for either Africanized or European queens (Table 6-5). Worker bee type also had no effect on daily egg laying rates of sister queens (Table 6-7).

Brood Production Rates during First Brood Cycle

The amounts of unsealed, sealed and total brood at day 12 and at day 17 for each of the experimental colonies are presented in Table 6-8. There was no significant effect of worker bee population type on brood production at either day 12 or day 17. When all colony treatments were combined, total brood produced at day 12 was significantly correlated with total brood produced at day 17 ($P<0.05$).

Changes in worker bee population for each colony are shown in Table 6-9. The estimated daily mortality rates for Africanized worker bees were not significantly different than those for European worker bees.

The numbers of unsealed brood, sealed brood and total brood at day 12 and day 17 expressed as percentages of the adult population are presented in Table 6-10. There was no significant difference between Africanized and European worker bees.

Sister queens are compared in Table 6-11. The performance of the European queen pair was similar with either Africanized and European worker bees. One sister of Africanized queen pair (A62) performed

better with Africanized bees, and one sister of Africanized queen pair (A57) performed better with European bees.

Egg laying rates for queens evaluated in the daily egg laying experiment were compared with estimated egg laying rates derived from the brood production experiment (Table 6-12). There was no correlation between daily egg laying rates with either the estimated daily egg laying rates for the first 12 days or the estimated daily egg laying rates for 17 days. Estimated daily egg laying rates at day 12 was significantly correlated to the estimated overall egg laying rates at day 17 ($P<0.05$).

Adequate pollen and nectar resources were available during the experiment. Each colony stored pollen and had an overall weight gain. There was no significant difference between colonies with Africanized or European worker bees with respect to pollen stored or weight gained. The amount of pollen stored by each colony was not significantly correlated with colony weight gain, total brood produced, or mean estimated daily mortality. Colony weight gain was positively correlated ($P<0.05$) with total brood produced and negatively correlated ($P<0.05$) with mean estimated daily mortality.

Discussion

The purpose of these experiments was to compare egg laying rates between Africanized and European queen honey bees and to determine if Africanized and European worker bees differentially affect brood production and/or the queen's egg laying behavior. Results from daily egg laying rates indicate that there was no significant difference between Africanized and European queens during the initial colony growth period under identical experimental conditions in Venezuela. There was

also no significant difference between Africanized and European worker bees on brood production rates or egg laying rates of either Africanized or European queens.

Differences in egg laying and brood production rates could be evaluated by eliminating potential differences in foraging success between Africanized and European bees by providing surplus honey to each experimental colony and by controlling comb cell size. Differences in brood production caused by differences in resource utilization efficiency as a result of either smaller bee size or increased foraging success were, therefore, not evaluated. For a given amount of food, a greater number of smaller, Africanized bees can be produced compared with larger, European bees (Fletcher and Tribe 1977a; Tribe and Fletcher 1977). However, this advantage for Africanized bees with respect to their smaller size, would only be present if resources were limited. Therefore, surplus honey was provided for each colony to reduce the effects of limited resources and the effects of differential foraging success between the two honey bee populations. Controlling comb cell size eliminated brood production differences based on bee size. For example, a given number of nurse bees may be able to rear more smaller bees than larger ones. In order that queen-worker bee interactions could be evaluated, European comb size was selected because of the difficulties both European queens and worker bees have with Africanized comb as discussed earlier.

Egg laying rates observed during these experiments were within the range reported for Africanized bees in French Guiana (Winston and Taylor 1980) but lower than the maximum reported for either population (Fletcher 1978; Ribbands 1953). Several factors can account for this

difference. First, the experimental colonies used in this study may have been only one-tenth the size of the managed, production colonies in which the maximum rates were observed. Because egg laying rates are correlated with the number of worker bees in the colony (Moeller 1958), the lower egg laying rates may have been a result of smaller colonies. Second, the intensity of colony disturbance, particularly during the daily egg laying experiment, would have reduced egg laying rates and increased egg and larval mortality. Third, egg laying rates for artificially inseminated queens may be lower than for naturally mated queens (Harbo and Szabo 1984). The purpose of this study was not to determine absolute egg laying rates but to compare egg laying and brood production rates for Africanized and European queens during the initial colony growth phase under identical conditions.

There were no differences in egg laying and brood production rates for Africanized and European bees during the initial colony growth phase. When colonies increase in size and approach their maximum growth phase and queens are maximally challenged, there may be a difference between Africanized and European queens and colonies. However, comparisons of queen pupal weights (as a correlate of egg laying rates) suggest that there would be no difference between Africanized and European queens with respect to potential egg laying capacity (Chapter V).

Colony growth rates and therefore reproductive rates are affected by two other demographic parameters: adult longevity and brood mortality. Winston and Katz (1981) found that European worker bees were longer lived than Africanized worker bees under identical conditions in Venezuela (26.3 compared with 22.7 days). This difference would give

European colonies a growth rate advantage. Unfortunately, brood mortality for both Africanized and European honey bees during the initial colony growth phase has not been investigated under identical conditions.

Based on the results from these studies and those evaluating other colony demographic parameters, e.g., worker development times (Chapter II) and queen maturation rates (Chapter IV), it must be concluded that differences in reproductive rates between Africanized and European honey bees in South America cannot be attributed to intrinsic demographic factors. Reproductive rates in honey bees are a function of at least two other factors--resource availability and resource utilization efficiency. Chapter VIII presents a hypothesis to explain the success of Africanized honey bees based on differences in resource utilization efficiency. This hypothesis is based on differences between Africanized and European bees with respect to foraging behavior, brood production efficiency as a function of bee size, and resource-induced absconding.

TABLE 6-1. Daily egg laying rates of Africanized and European queens with European nurse bees, trial 1.

	ONE-DAY HIGH	MEAN ± SD (n)
AFRICANIZED QUEENS		
A57 (W42)	550	473.2 ± 68.5 (6)
A62 (W81)	857	825.0 ± 47.0 (3)
EUROPEAN QUEENS		
YK (Y4)	951	922.0 ± 41.0 (2)
YD5 (Y42)	763	689.6 ± 66.4 (5)
GK30 (Y63)	710	636.7 ± 80.8 (3)
GK30 (Y64)	705	575.6 ± 101.3 (5)
ANALYSIS[a]		NS

[a]Mann-Whitney U test, two-tailed, alpha = 0.05; evaluated for all samples (n = 24).

TABLE 6-2. Daily egg laying rates of Africanized and European queens with European nurse bees, trial 2.

	ONE-DAY HIGH	MEAN ± SD (n)
AFRICANIZED QUEENS		
A57 (W42)	740	699.8 ± 39.5 (4)
A62 (W81)	953	915.8 ± 30.7 (4)
EUROPEAN QUEENS		
GK30 (Y63)	779	736.8 ± 32.2 (4)
ANALYSIS[a]		NS

[a]Mann-Whitney U test, two-tailed, alpha = 0.05; evaluated for all samples (n = 12).

TABLE 6-3. Daily egg laying rates of Africanized and European queens with Africanized nurse bees, trial 2.

	ONE-DAY HIGH	MEAN ± SD (n)
AFRICANIZED QUEENS		
A61 (W61)	847	799.5 ± 67.2 (2)
A57 (W41)	725	694.0 ± 39.1 (4)
A62 (W85)	883	798.7 ± 92.3 (3)
EUROPEAN QUEENS		
GK30 (Y61)	850	823.5 ± 37.0 (4)
YD5 (Y52)	949	782.7 ± 231.7 (3)
ANALYSIS[a]		NS

[a]Mann-Whitney U test, two-tailed, alpha = 0.05; evaluated for all samples (n = 16).

TABLE 6-4. Effect of nurse bee genotypes on the daily egg laying rates of Africanized and European queens, trial 2.

	MEAN ± SD (n)	
AFRICANIZED NURSE BEES		
AFRICANIZED QUEENS		
A61 (W61)	799.5 ± 67.2 (2)	
A57 (W41)	694.0 ± 39.1 (4)	
A62 (W85)	798.7 ± 92.3 (3)	
COMBINED	752.3 ± 79.6 (9)	(A)
EUROPEAN QUEENS		
GK30 (Y61)	823.5 ± 37.0 (4)	
YD5 (Y52)	782.7 ± 231.7 (3)	
COMBINED	806.0 ± 138.0 (7)	(B)
COMBINED AFRICANIZED AND EUROPEAN QUEENS	775.8 ± 108.4 (16)	(C)
EUROPEAN NURSE BEES		
AFRICANIZED QUEENS		
A57 (W42)	699.8 ± 39.5 (4)	
A62 (W81)	915.8 ± 30.7 (4)	
COMBINED	807.8 ± 120.0 (8)	(D)
EUROPEAN QUEEN		
GK30 (Y63)	736.8 ± 32.2 (4)	(E)
COMBINED AFRICANIZED AND EUROPEAN QUEENS	784.1 ± 103.3 (12)	(F)

TABLE 6-5. Analyses of the effect of queen genotypes and nurse bee genotypes on the daily egg laying rates of Africanized and European honey bees. Letters represent the different treatments presented in Table 6-4. Mann-Whitney U test, two-tailed, alpha = 0.05 (Siegel 1956).

H1: There is no difference in egg laying rates between Africanized and European queens.

i.	Africanized nurse bees A x B	NS
ii.	European nurse bees D x E	NS
iii.	Africanized and European nurse bees combined (A + D) x (B + E)	NS

H2: There is no differential effect of Africanized and European worker bees on egg laying rates.

i.	Africanized queens A x D	NS
ii.	European queens B x E	NS
iii.	Africanized and European queens combined C x F	NS

TABLE 6-6. Daily egg laying rates of Africanized and European queens; comparison between genotypes within each population.

	MEAN + SD (n)	ANALYSES
AFRICANIZED QUEENS		
1. TRIAL 1--EUROPEAN NURSE BEES		
A57 (W42)	473.2 ± 68.5 (6)	
A62 (W81)	825.0 ± 47.0 (3)	
		P<0.05[a]
2. TRIAL 2--EUROPEAN NURSE BEES		
A57 (W42)	699.8 ± 39.5 (4)	
A62 (W81)	915.8 ± 30.7 (4)	
		P<0.05[a]
3. TRIAL 2--AFRICANIZED NURSE BEES		
A61 (W61)	799.5 ± 67.2 (2)	
A57 (W41)	694.0 ± 39.1 (4)	
A62 (W85)	798.7 ± 92.3 (3)	
		NS[b]
4. COMBINING #2 AND #3		P<0.05[b]
EUROPEAN QUEENS		
1. TRIAL 1--EUROPEAN NURSE BEES		
YK (Y4)	922.0 ± 41.0 (2)	*[c]
YD5 (Y42)	689.6 ± 66.4 (5)	
GK30 (Y63)	636.7 ± 80.8 (3)	
GK30 (Y64)	575.6 ± 101.3 (5)	
		NS[b]
2. TRIAL 2--EUROPEAN NURSE BEES		
GK30 (Y63)	736.8 ± 32.2 (4)	
3. TRIAL 2--AFRICANIZED NURSE BEES		
GK30 (Y61)	823.5 ± 37.0 (4)	
YD5 (Y52)	782.7 ± 231.7 (3)	
		NS[a]
4. COMBINING #2 AND #3		NS[b]

[a]Mann-Whitney U test, two-tailed, alpha = 0.05.
[b]Kruskal-Wallis one-way analysis of variance by ranks, alpha = 0.05.
[c]YK (Y4) was significantly different, P<0.05, from other genotypes in the group when evaluated by pairs using the Mann-Whitney U test, two-tailed, alpha = 0.05.

TABLE 6-7. Comparison of daily egg laying rates for sister queens with Africanized and European nurse bees. Mean ± SD (n = sample size).

	AFRICANIZED NURSE BEES	EUROPEAN NURSE BEES	ANALYSES[a]
AFRICANIZED QUEENS			
A57 (W41)	694.0 ± 39.1 (4)	---	NS
A57 (W42)	---	699.8 ± 39.5 (4)	
A62 (W85)	798.7 ± 92.3 (3)	---	NS
A62 (W81)	---	915.8 ± 30.7 (4)	
EUROPEAN QUEENS			
GK30 (Y61)	823.5 ± 37.0 (4)	---	NS
GK30 (Y63)	---	736.8 ± 32.2 (4)	

[a]Mann-Whitney U test, two-tailed, alpha = 0.05.

TABLE 6-8. Comparison of brood production in cm^2 for Africanized and European queens during two periods of the first brood cycle.

	DAY 12			DAY 17		
	USB[a]	SB[b]	TB[c]	USB	SB	TB
AFRICANIZED NURSE BEES						
AFRICANIZED QUEENS						
A62 (W81)	1053	962	2015	1484	1301	2785
A57 (W42)	416	666	1082	493	818	1311
EUROPEAN QUEEN						
GK30 (Y63)	1775	819	2594	1330	1756	3086
EUROPEAN NURSE BEES						
AFRICANIZED QUEENS						
A62 (W85)	906	823	1729	434	1354	1788
A57 (W41)	1481	487	1968	1018	1256	2274
EUROPEAN QUEEN						
GK30 (Y61)	1614	728	2342	1580	1519	3099
ANALYSES[d]						
AFR WORKERS x EUR WORKERS	NS	NS	NS	NS	NS	NS

[a]USB = unsealed brood (eggs and larvae).
[b]SB = sealed brood (pre-pupae and pupae).
[c]TB = total brood.
[d]Mann-Whitney U test, one-tailed, alpha = 0.05. AFR = Africanized; EUR = European.

TABLE 6-9. Colony adult population changes during first brood cycle.

	DAY 0[a]	DAY 12[b]	DAY 17[c]	ESTIMATED DAILY MORTALITY RATE[d]
AFRICANIZED NURSE BEES				
AFRICANIZED QUEENS				
A62 (W81)	10,169	4,850	2,634	443
A57 (W42)	10,169	4,408	2,007	480
EUROPEAN QUEEN				
GK30 (Y63)	10,169	6,511	4,987	305
				MEAN = 409
EUROPEAN NURSE BEES				
AFRICANIZED QUEENS				
A62 (W85)	10,435	5,375	3,267	422
A57 (W41)	10,435	6,102	4,297	361
EUROPEAN QUEEN				
GK30 (Y61)	10,435	6,321	4,607	343
				MEAN = 375
ANALYSIS[e]				NS

[a]Population at Day 0 was estimated as described in methods.
[b]Population at Day 12 estimated by: Day 0 - [(Day 0 - Day 17)(12/17)].
[c]Population at Day 17 was estimated as described in methods.
[d](Day 0 - Day 17)/17.
[e]Mann-Whitney U test, one-tailed, alpha = 0.05.

TABLE 6-10. Brood production during first brood cycle expressed as a percent of adult population.

	DAY 12			DAY 17		
	USB[a]	SB[b]	TB[c]	USB	SB	TB
AFRICANIZED NURSE BEES						
AFRICANIZED QUEENS						
A62 (W81)	92.3	84.3	176.6	239.4	209.9	449.3
A57 (W42)	40.1	64.2	104.3	104.4	173.2	274.6
EUROPEAN QUEEN						
GK30 (Y63)	115.9	53.4	169.3	113.3	149.6	262.9
EUROPEAN NURSE BEES						
AFRICANIZED QUEENS						
A62 (W85)	71.6	65.1	136.7	56.4	176.1	232.5
A57 (W41)	103.2	33.9	137.1	100.7	124.2	224.9
EUROPEAN QUEEN						
GK30 (Y61)	108.5	48.9	157.4	145.8	140.1	285.9
ANALYSES[d]						
AFR WORKERS x EUR WORKERS	NS	NS	NS	NS	NS	NS

[a]Unsealed brood (eggs and larvae); colony adult population estimated for Day 12 and Day 17 as described in methods.
[b]Sealed brood (pre-pupae and pupae).
[c]Total brood.
[d]Mann-Whitney U test, one-tailed, alpha = 0.05. AFR = Africanized; EUR = European.

TABLE 6-11. Comparison of overall egg laying rates between Africanized and European sister queens with Africanized and European nurse bees during the first brood cycle.

	AFRICANIZED NURSE BEES[a]	EUROPEAN NURSE BEES
AFRICANIZED QUEENS		
A62 (W81)	696	--
A62 (W85)	--	447
A57 (W42)	328	--
A57 (W41)	--	568
EUROPEAN QUEENS		
GK30 (Y63)	772	--
GK30 (Y61)	--	775

[a]Overall egg laying rate = $(TB_{17}/17)(4.25)$.

Table 6-12. Comparison of egg laying rates during daily egg laying rate experiment and brood production experiment.

	AFRICANIZED NURSE BEES			EUROPEAN NURSE BEES		
QUEENS	ELR[a]	BP12[b]	BP17[c]	ELR	BP12	BP17
AFRICANIZED						
A62 (W81)	--	714	696	916	--	--
A62 (W85)	799	--	--	--	612	447
A57 (W42)	--	383	328	700	--	--
A57 (W41)	694	--	--	--	697	568
EUROPEAN						
GK (Y63)	--	918	772	737	--	--
GK (Y61)	824	--	--	--	829	775
ANALYSES[d]	ELR x BP12		NS			
	ELR x BP17		NS			
	BP12 x BP17		P<0.05			

[a]Daily egg laying rate experiment, means.
[b]Brood production experiment; egg laying rate estimated for first 12 days of brood cycle by dividing total brood at day 12 by 12.
[c]Brood production experiment; egg laying rate estimated for first 17 days of brood cycle by dividing total brood at day 17 by 17.
[d]Spearman rank correlation, alpha = 0.05; Africanized and European nurse bees combined.

CHAPTER VII
SUCCESSFUL HYBRIDIZATION BETWEEN AFRICANIZED AND EUROPEAN HONEY BEES IN VENEZUELA WITH IMPLICATIONS FOR NORTH AMERICA

Introduction

In 1956 African honey bees, Apis mellifera scutellata, formerly classified as A. m. adansonii (Ruttner 1976a, 1976b, 1981), were imported into southeastern Brazil (Kerr 1967). Their hybridized descendents, known as Africanized honey bees (Goncalves 1982), have rapidly spread throughout tropical South and Central America as far north as Honduras and El Salvador (Rinderer 1986). The dispersion from their original importation site into new areas has been rapid--200-500 km per year (Taylor 1977, 1985; Winston 1979a). As Africanized honey bees have dispersed into new areas, they have rapidly increased in number (Otis 1982a) and have attained dramatic population densities (Michener 1975): 4-8 colonies/km^2 (Taylor 1985), or as high as 107.5 colonies/km^2 in the cerrado habitats in the Brazilian states of Goias and Mato Grosso (Kerr 1971 cited in Michener 1975). There are now probably more than ten million feral colonies of Africanized honey bees in South and Central America (Winston, Taylor and Otis 1983). Their success in these habitats, compared with European populations of honey bees, may be attributed to their foraging behavior which is more suited to the resource patterns of the tropics (Nunez 1973, 1979a, 1982; Rinderer, Bolten, Collins and Harbo 1984; Rinderer, Collins and Tucker 1985; Winston and Katz 1982). As a result of both their foraging

success and length of time throughout the year that resources are available in the tropics, Africanized honey bees have a high annual reproductive rate, which is responsible for both their rate of dispersal into new areas and high colony densities. Net reproductive rates for Africanized bees have been estimated to be 16 colonies per colony per year based on demographic data collected in French Guiana (Otis 1980, 1982a) compared with 0.92-0.96 (Seeley 1978) or 3-3.6 when afterswarms are counted (Winston 1980a; Winston, Taylor and Otis 1983) for European honey bees in North America.

Particularly in the region of their importation (southeastern Brazil), there has been ample opportunity for hybridization with both managed and feral European honey bees [primarily A. m. mellifera and A. m. ligustica which had been imported into Brazil by 1845 (Gerstaker cited in Pellet 1938; Woyke 1969)]. However, despite opportunity for hybridization, Africanized honey bees have maintained behavioral, chemical and morphological characteristics similar to their African parental population and distinguishable from European honey bees: colony defense behavior (=stinging behavior) (Collins, Rinderer, Harbo and Bolten 1982; Stort 1974, 1975a, 1975b, 1975c, 1976); reproductive rates (Fletcher 1978; Fletcher and Tribe 1977a; Otis 1980, 1982a); absconding behavior (reviewed by Fletcher 1978; Winston, Otis and Taylor 1979; Winston, Taylor, and Otis 1983); foraging and hoarding behavior (Nunez 1973, 1979a, 1982; Rinderer, Bolten, Collins and Harbo 1984; Rinderer, Bolten, Harbo and Collins 1982; Rinderer, Collins and Tucker 1985; Winston and Katz 1982); worker bee longevity (Winston and Katz 1981); development times (Chapters II and IV; Harbo, Bolten, Rinderer and Collins 1981); selection preferences for nest cavity sizes

(Rinderer, Collins, Bolten and Harbo 1981; Rinderer, Tucker and Collins 1982); allozyme patterns (Nunamaker and Wilson 1981; Sylvester 1982); cuticular hydrocarbon composition (Carlson and Bolten 1984, and unpublished data); adult bee size and comb cell size (Chapter III; Michener 1975); and morphometric relationships (Daly and Balling 1978).

This apparent lack of evidence for hybridization has been attributed primarily to some degree of reproductive isolation between the Africanized and European populations (Kerr and Bueno 1970; Taylor 1985). Three isolating mechanisms have been suggested: assortative mating (Kerr and Bueno 1970); physiological incompatibility with respect to the drone ejaculation response (Kerr and Bueno 1970); and differences in drone and presumably the queen flight times between the Africanized and European populations (Taylor 1985; Taylor, Kingsolver and Otis in press). At best, these mechanisms may be only partially effective and are not likely to account for the apparent lack of hybridization. For example, Kerr and Bueno (1970) present data to support assortative mating even though 35% and 42% of the matings evaluated were hybrid. With respect to differences in queen and drone flight times, data from Venezuela suggest that mean peak drone flight times for Africanized and European populations are separated by only 23 minutes and that drones from both populations are present in the mating areas at all times during the approximately three hour flight period (Taylor, Kingsolver and Otis in press).

A more probable argument for the maintenance of the African characteristics is based on selection advantages for the African genotype in tropical habitats of South America, which are characterized by resource distribution patterns similar to the ones in Africa where

these bees evolved. Two lines of evidence support this selectionist argument. First, the foraging behavior of the Africanized honey bees, characterized by solitary foraging and less colony recruitment, would be more adaptive in tropical areas with rich, but dispersed, resources (Rinderer, Bolten, Collins and Harbo 1984; Rinderer, Collins and Tucker 1985). Second, there is much historic evidence that European honey bees have not been successfully maintained (probably due to starvation) in many areas of tropical South America that are now densely populated with Africanized honey bees (Bolten, personal observation; Michener 1972; Winston, Taylor and Otis 1983).

The selectionist argument allows for hybridization between Africanized and European populations, with the African genotype being selected for under the physical and biotic parameters characteristic of tropical areas. Selection for the African genotype would then account for the present population in South and Central America being behaviorally, chemically and morphologically similar to the African parental type.

These two alternative hypotheses for the maintenance of the African parental characteristics--reproductive isolation between the Africanized and European honey bee populations versus selection for the African genotype in tropical regions--suggest different scenarios for the potential impact of Africanized honey bees in North America. One scenario resulting from reproductive isolation would limit Africanized honey bees in their northern movement because of their inability to overwinter (Taylor 1985; Taylor and Spivak 1984). This is based on the observation that the parental African population as well as Africanized bees do not have the thermoregulatory capabilities to survive the cold

temperatures of temperate winters (Nunez 1979b; Woyke 1973). The other scenario based on successful hybridization and resultant genetic introgression would have the stinging behavior of the Africanized honey bees and the potential public health hazard widespread in North America and not limited to the southern regions.

A comparison between hybrid and non-hybrid mating success is lacking for the Africanized and European populations of honey bees. Although reciprocal F1 crosses (Africanized queen x European drone; European queen x Africanized drone) can be successfully produced with artificial insemination (Chapater III), natural matings have been difficult to evaluate because of the inability to distinguish between hybrid and non-hybrid progeny on the individual level. It is not possible to determine which drones have mated a queen because honey bees mate in the air away from the hive, and there are no available genetic markers in the Africanized population. Identifying individual honey bees as either Africanized or European is not presently possible (Carlson and Bolten 1984; Page and Erickson 1985; Rinderer and Sylvester 1981). However, there is promise that DNA analyses will allow individuals to be identified (Hall in press).

This paper evaluates an important parameter with respect to the question of successful hybridization between Africanized and European honey bees: the mating success of both Africanized and European queens with Africanized drones. The mating success of European queens x Africanized drones was compared with the mating success of Africanized queens x Africanized drones in an isolated area in Venezuela with no European honey bees but with a feral population of Africanized honey bees. Because of present legal restrictions, Africanized bees cannot be

taken into areas with only European honey bees so the reciprocal cross was not evaluated. However, evaluating the success of the European queen x Africanized drone cross is important because it represents the most probable initial hybridization that will occur when Africanized honey bees invade North America (Mexico and U.S.A.).

Methods

The Africanized queen mother (A26) was removed from a feral colony in eastern Venezuela where there were no known European honey bees. The colony was identified as Africanized by its behavior and small comb cell size characteristic of the Africanized population (4.5-5.0 mm between opposite sides of the hexagon, Chapter III). The European queen mother (L13) was produced by a commercial queen producer in the southeastern U.S.A. and shipped to Venezuela.

Experimental queens were produced from each queen mother by the standard queen rearing technique of transferring (grafting) 12 to 18-hour-old larvae into artificial queen cells that were then introduced into queen-cell producing colonies (Laidlaw 1979). After the queen cells were sealed, each cell was protected by a wire mesh cylinder (mesh size = 3.0 mm). Virgin queens were allowed to emerge in the cell-producing colonies. Newly emerged virgins were marked for identification and then stored in a strong, queenless colony. The following day they were introduced into individual, five-frame colonies with Africanized worker bees from which natural matings could occur. Queens were released into these mating colonies using standard three-hole mailing cages (Laidlaw 1979). Based on earlier calculations, natural release from the mailing cages was estimated to take 2.5-3.0 days. Marked, virgin queens were introduced into mating colonies rather

than mature queen cells so that the identity of the experimental queens would later be certain. The mating colonies were located in an area in eastern Venezuela where there was no known European honey bees, but which was densely populated with feral Africanized colonies.

Eighteen days after introduction into mating colonies, the queens were collected, and the spermatazoa in their spermatheca were counted using hemacytometers (Mackensen and Roberts 1948; Mackensen and Tucker 1970). The criterion for mating success was the number of spermatozoa in the spermatheca. In addition, the age of the queen when oviposition first started was calculated by determining the age of the oldest brood in each colony.

Results

Numbers of spermatozoa counted in the spermatheca of Africanized and European queens are summarized in Table 7-1. The mean number of spermatozoa in Africanized queens (4.09 $\pm$ 0.50 million) was not different from the mean number in European queens (4.12 $\pm$ 0.58 million; t-test, two-tailed, alpha = 0.05). European queens began oviposition on the 10th day post-emergence, one day sooner than the Africanized queens ($P<.001$; Kolmogorov-Smirnov two-tailed test, chi-square distribution, df = 2). The time from adult emergence to initiation of oviposition reported here (Table 7-1) is longer than the maturation interval reported in Chapter IV, which may be a result of having introduced virgin queens into the mating colonies rather than mature queen cells. There was no correlation between the time post-emergence to initiation of oviposition and the number of spermatozoa in the spermatheca (Spearman's rank correlation coefficient, two-tailed, alpha = 0.05). The acceptance of the Africanized and the European virgin queens

introduced into the mating nuclei was 50% and 61%, respectively. There was no significant difference in acceptance (Fisher's exact probability test, alpha = 0.05).

Discussion

Evidence for Hybridization

There appears to be no effective reproductive isolating mechanism operating between the Africanized and European populations. The mating of both Africanized and European queens with Africanized drones was equally successful as judged by the number of spermatozoa in the spermatheca. Offspring from the hybrid crosses were viable with no apparent difference in mortality as determined by the uniformity of the brood pattern. Kerr and Bueno (1970) report that there may be a difference in ejaculation response between Africanized and European drones that may provide a potential isolating mechanism. Even if this exists, European queens were still able to successfully mate with Africanized drones without any apparent problem, as determined by both the numbers of spermatozoa in their spermatheca and the age when oviposition began.

Although the same queen pheromone is produced by three sympatric Asiatic species of Apis (Butler, Calam and Callow 1967; Shearer, Boch, Morse and Laigo 1970), reproductive isolation occurs between the three species because there is no overlap in times of drone flight (Koeniger and Wijayagunasekera 1976). The situation between Africanized and European populations of honey bees is quite different with respect to the time of flight of the queens and drones. Data from Venezuela show that flight times for Africanized and European drones completely overlap during the approximately three hours of mating flight activity with only

23 minutes separating the mean times of peak flight activity for each population (Taylor, Kingsolver and Otis in press). This difference does not provide a satisfactory mechanism for reproductive isolation between the Africanized and European honey bee populations--particularly because any unfavorable climatic conditions, e.g., high winds, cloudiness, high humidity, or rain (Gary 1975), cause mating flights of reproductives from both populations to more completely converge to times of favorable weather conditions. The data on reproductive success (determined by the number of spermatozoa in the spermatheca) of European queens mating with Africanized drones presented in this study demonstrate that any differences in mean peak flight times did not effectively prevent hybridization of European queens with Africanized drones.

Evidence that extensive hybridization has already occurred between the introduced African honey bees and the previously established European honey bees can be demonstrated by the increase in genetic diversity in the Africanized population. For example, Adams, Rothman, Kerr and Paulino (1977) concluded that the large increase in number of sex alleles in the population of honey bees in southeastern Brazil is a result of hybridization between African and European honey bees. Page and Erickson (1985) also suggest that hybridization has occurred based on the variation in behavior and appearance of Africanized bees in Venezuela.

Impact of Hybridization

Demonstration that successful hybridization can and does occur has important implications with respect to the potential impact the Africanized honey bee will have in North America. First is the negative impact that hybridization would have. The parental African population

as well as Africanized bees may not have the thermoregulatory capabilities to survive the cold temperatures of temperate winters (Nunez 1979b; Woyke 1973). Based on the temperature limits of the parental population, Taylor (1985) and Taylor and Spivak (1984) predicted the northern limits of Africanized honey bees in North America. However, Africanized honey bees may acquire, through hybridization with European honey bees in Mexico and southern U.S.A., the ability to overwinter farther north than is presently expected. That is, the overwintering genome of the European honey bees may become introgressed into the Africanized genome. Or, the corollary, the stinging behavior characteristic of the Africanized honey bees may become introgressed into the overwintering European population. Successful genetic introgression of these traits may not be a rapid process because these traits are polygenic and/or may involve coadapted genomes. However, because hybridization occurs, the potential for successful genetic introgression exists and must be considered. Hybridization may, therefore, result in the stinging behavior of the Africanized honey bees becoming a potential public health hazard throughout North America, not just in the warmer southern regions. That this may be the unfortunate outcome of hybridization is supported by recent investigations in Argentina, which have demonstrated that Africanized honey bees are distributed farther south than predicted based on temperature limits of the parental population (Dietz, Krell and Eischen 1985; Krell, Dietz and Eischen 1985).

The U.S. Department of Agriculture Economic Research Service has recently evaluated the potential impact of Africanized honey bees in the U.S.A. (McDowell 1984). Unfortunately this report does not consider the

possibility that further hybridization between Africanized and European honey bees might result in the stinging behavior of Africanized honey bees becoming established throughout the northern regions of the U.S.A. The economic consequences as well as public health hazards may be more widespread throughout North America than previously thought. For any solutions to the Africanized honey bee problem to be successful, a realistic assessment of the potential problem is necessary.

However, hybridization may also have a positive impact. Coupled with selection favoring both the foraging and thermoregulatory behavior of European honey bees in temperate regions, hybridization between Africanized and European bees may have the positive effect of increasing the rate at which African genes become rare in the population. There is a large population of European honey bees, both managed and feral, in North America (perhaps greater than 15 million colonies in the U.S.A. alone), which is particularly dense in the south where the Africanized bees will first enter the U.S.A. The invading Africanized population would be quite small relative to the existing European population, increasing the frequency of hybridization and resultant "swamping" (or diluting) of African genes.

The problem of Africanized honey bees may be reduced prior to their entry into the U.S.A. because of both the potential for hybridization and competition for available floral resources with European honey bees in Mexico. Mexico has a larger population of European honey bees than any other country in Latin America. When Africanized honey bees enter Mexico, they will be entering a region that already has an extensive, established population of European honey bees, both managed and feral [2.6 million managed colonies alone (Zozaya cited in Taylor 1985)].

Competition with an established population of honey bees for limited floral nectar and pollen resources will be much greater than Africanized bees have previously experienced in any areas in South America. This competition will greatly slow their dispersal. In many areas of Mexico and southern U.S.A., pollen and nectar resources are already close to being saturated by the existing honey bee population. In addition, under temperate resource conditions, the foraging behavior of Africanized honey bees (which is more appropriate to tropical resource patterns) will be at a disadvantage relative to the foraging behavior of the European honey bee population, which is characterized by greater colony recruitment (Rinderer, Bolten, Collins and Harbo 1984; Rinderer, Collins and Tucker 1985; Visscher and Seeley 1982).

The selective advantage of the foraging and/or thermoregulatory behavior of European honey bees has been demonstrated in temperate regions. African honey bee queens were introduced into North America during the late 1800's and early 1900's when the beekeeping industry in the U.S.A. was developing (Morse et al. 1973) and more recently, into Louisiana (Cantwell 1974; Morse et al. 1973; Taber 1961). However, due to hybridization and selection against the African genotype, the impact of these introductions of African bees is undetectable today. There have also been unsuccessful introductions of African and Africanized bees into Europe (Cantwell 1974; Morse et al. 1973; Woyke 1973). Therefore, these factors--a large, established population of European honey bees, and both a foraging and thermoregulatory behavior in European bees better adapted to temperate conditions--precludes using South and Central America as a model for North America in predicting the impact, as well as the rate of spread, of Africanized honey bees.

Kin recognition has been suggested as another mechanism that may help preserve the African genotype in the hybridized honey bee population in South and Central America (Hall in press). However, data presented in Table 4-2 demonstrate that this is unlikely because both Africanized and European worker bees reared both Africanized and European queens with equal frequency.

There have been several suggestions that Africanized drones may have a mating advantage over European drones (Kerr and Bueno 1970; Michener 1975; Morse 1984; Rinderer 1986; Rinderer, Hellmich, Danka and Collins 1985; Taylor 1985). Taylor (1985) suggests that this mating advantage would reduce hybridization. On the contrary, a mating advantage for Africanized drones would increase the rate of hybridization between Africanized and European honey bee populations. When Africanized honey bees begin invading North America (Mexico and U.S.A.), they will be greatly outnumbered by the established European honey bee population. With a mating advantage, the frequency of European queen x Africanized drone matings would be greater than expected based solely on the relative frequency of each population, thereby resulting in greater hybridization.

Unfortunately, Africanized honey bee research has been characterized by conceptualizing the Africanized honey bee as a distinct entity that is reproductively isolated (Taylor 1977, 1985; Taylor and Spivak 1984) rather than as a population within a species fully capable of hybridization. Clearly, the Africanized honey bee is not a species invading a new habitat (North America) that is free of competition from conspecifics. Thus, the spread of Africanized bees (African genes) in temperate North America will be: 1) farther north than predicted by the

geographic limits of the parental population because of hybridization and resultant genetic introgression; 2) slowed considerably by competition for available resources by an established population of European honey bees; 3) swamped through hybridization with a more numerous, established population of European honey bees; 4) at a disadvantage with respect to foraging behavior; and 5) limited by selection against those colonies that have not acquired through hybridization the ability to overwinter.

There has been a general lack of support for the selectionist argument for the maintenance of the African genotype in favor of the hypothesis of reproductive isolation. With the demonstration that hybridization is successful, coupled with the recent observations of the distribution patterns of Africanized honey bees in Argentina (Dietz, Krell and Eischen 1985; Krell, Dietz and Eischen 1985), further consideration of the selectionist argument for the maintenance of the African characteristics in the Africanized honey bee in South and Central America is necessary.

TABLE 7-1. Mating success of Africanized and European honey bee queens.

	TIME TO OVIPOSITION[a]	NO. SPERM ($\times 10^6$)[b]	CORRELATIONS[c]
AFRICANIZED GENOTYPE (A26)	11 (8)	4.09 $\pm$ 0.50 (8)	NS
EUROPEAN GENOTYPE (L13)	10 (11)	4.12 $\pm$ 0.58 (11)	NS
ANALYSES	$P<0.001$[d]	NS[e]	

[a]Median days post-emergence to initiation of oviposition (sample size). One-day-old virgins were introduced into mating colonies.
[b]Mean $\pm$ SD (sample size) of spermathecal spermatozoa number.
[c]Spearman's rank correlation coefficient, two-tailed, alpha = 0.05.
[d]Kolmogorov-Smirnov two-tailed test, chi-square distribution, df = 2.
[e]t-test, two-tailed, alpha = 0.05.

CHAPTER VIII

DISCUSSION: FACTORS CONTRIBUTING TO THE SELECTION ADVANTAGE OF AFRICANIZED HONEY BEES IN SOUTH AMERICA-- THE RESOURCE UTILIZATION EFFICIENCY HYPOTHESIS

Success of Introduced Populations of Honey Bees

Thirty years ago African honey bees, Apis mellifera scutellata [formerly classified as A. m. adansonii (Ruttner 1976a, 1976b, 1981)], were introduced into southeastern Brazil (Kerr 1967). Offspring, known as Africanized honey bees because of hybridization with European honey bees (Goncalves 1982), have rapidly dispersed throughout South America, sometimes achieving dramatically high population densities (Michener 1975; Taylor 1977, 1985). In 1982 Africanized honey bees entered Panama (Buchmann 1982) and by 1986 were as far north as Honduras and El Salvador (Rinderer 1986). The success and biological impact of Africanized honey bees in these tropical and sub-tropical regions, compared with the lack of success of European honey bees in these same regions, is a result of a selection advantage for the Africanized (= African) genotype in tropical resource and climatic conditions. The difference in success between Africanized and European honey bees is evidenced by the fact that

> European bees in Brazil were never commonly found living wild in the forests and countryside. This was especially true in tropical forest regions, where honey bees were virtually restricted to a few apiaries...Everyone questioned on the matter emphasized the increase in bees away from apiaries that occurred with the arrival of the Brazilian [Africanized] bees. (Michener 1972, p. 15).

The selection advantage for Africanized bees may be a result of behavioral and/or physiological characteristics that may include differences in resource utilization and/or colony demography. It is not surprising that Africanized honey bees are better adapted to tropical conditions than are European honey bees, considering the former are derived from imported African bees that evolved under similar tropical and sub-tropical conditions in Africa. Fletcher (1978) has reviewed the biological characteristics of the parental population of African honey bees in Africa.

The spread and impact of Africanized honey bees in South America must, however, be kept in perspective. European honey bees introduced into North America early in the 17th century (Pellett 1938) dispersed throughout North America, also achieving high population densities. In general, honey bees are very successful not only in their native habitats but in almost every region where they have been introduced. Their success is based on a highly developed social system that allows honey bees to: 1) develop large, perennial colonies that are able to buffer climatic changes; 2) efficiently utilize resources because of advanced communication and recruitment systems; and 3) defend against both vertebrate and invertebrate predators because of their very effective colony defense behavior.

The question with which we are concerned in these studies is not what makes A. mellifera more successful than other species nor what impact introduced honey bees have on native pollinator communities (see Roubik 1978, 1979, 1980, 1982, 1983; Roubik and Buchmann 1984). Nor is it a question of comparing Africanized honey bees in tropical regions with European honey bees in temperate regions (see Winston, Dropkin and

Taylor 1981 and Winston, Taylor and Otis 1983). Rather, the question is: what are the differences between Africanized and European populations of A. mellifera that make Africanized honey bees more successful in tropical regions not only in South America but also in Africa? European honey bees have not been successfully introduced into tropical areas of Africa despite numerous attempts (Fletcher 1977b, 1978).

Factors Affecting Honey Bee Reproductive Rates

The success of Africanized honey bees in South America--as judged by their rate of dispersal and their population densities (Michener 1975; Taylor 1977, 1985)--must surely be a result of a high reproductive rate. What are the differences between Africanized and European honey bees that allow for high reproductive rates in Africanized bees, and can these differences account for the impact of Africanized honey bees? This question does not involve identification or analysis of the proximal factors that are responsible for initiating reproduction, but does involve analysis of the components that affect the rate of reproduction.

Reproductive rates in honey bees are a result of an interaction of at least three factors, all of which affect colony growth rates: resource availability, resource utilization efficiency (foraging success, brood production efficiency, and bee size), and colony demographic parameters (primarily queen fecundity and adult worker bee longevity). Therefore, in order to evaluate reproductive differences between Africanized and European honey bee populations, all three factors need to be considered. Unfortunately, early research evaluated reproductive rates of Africanized honey bees by comparing data for

Africanized honey bees from South America with data from studies of European honey bees from North America, which not only were collected under different resource conditions but also different experimental conditions (Otis 1980, 1982a; Winston 1979b, 1980a; Winston, Dropkin and Taylor 1981).

Results from these earlier studies characterized the Africanized population as one with a dramatically high annual colony reproductive rate--four to five times greater than European honey bees in temperate regions (Otis 1980, 1982a; Winston 1980a; Winston, Taylor and Otis 1983). However, because these comparisons were not based on data collected under similar environmental or experimental conditions, they are inappropriate comparisons and cannot be used to identify either the factors responsible for the difference in reproductive rates between the two populations or the factors responsible for the success of Africanized honey bees. Because the experimental conditions were different (e.g., hive volume), these comparisons were also inappropriate for comparing temperate and tropical honey bee populations. Were the apparent differences in colony reproduction between the two honey bee populations the result of differences in: 1) colony demography; 2) environmental and climatic factors; 3) experimental design; 4) resource utilization efficiencies; or 5) some combination of factors? Are there intrinsic differences between the two honey bee populations with respect to colony demographic parameters that allow for a more rapid colony growth rate and result in a greater reproductive rate for the Africanized honey bee population? Or, are the differences in reproductive rates a result of climatic conditions and/or resource availabilities and utilization in the tropics compared with temperate

regions? Finally, were the relatively high reproductive rates observed for Africanized honey bees in these studies (Otis 1980, 1982a) simply an artifact of experimental conditions, particularly with respect to brood-nest crowding?

Brood-nest crowding is a primary stimulus for reproductive swarming in honey bees (Baird and Seeley 1983; Simpson 1966, 1973; Simpson and Riedel 1963). However, the experimental conditions affecting brood-nest crowding for Africanized colonies in South America were significantly different from the experimental conditions for European colonies in North America: the nest cavity volume for Africanized colonies was 22 liters (Otis 1980; Winston 1979b) compared with 42 liters for European colonies in North America (Winston 1980a). Despite these problems with respect to making valid comparisons, the earlier studies (particularly Winston 1979b) leave one with the impression that the apparent differences in reproductive rates between the two populations were primarily due to differences in demographic parameters and not to differences in environmental and experimental conditions, resource utilization, or some combination of factors.

What is the consequence of comparing reproductive rates of Africanized honey bees in South America with those of European honey bees in North America without considering differences in environmental conditions? Certainly, environmental conditions in temperate regions impose strict limits on the length of the reproductive (= swarming) season for honey bees because of a reduced growing season when floral resources (nectar and pollen) are available. The honey bee reproductive season is significantly shorter than the growing season, because colonies first have to grow to reproductive size before swarming can

occur. In addition, offspring (swarms) need a rather long period of time to grow and to hoard necessary surplus honey, while there are still floral resources available, in order to prepare for winter.

Most mortality of honey bee colonies in temperate areas occurs primarily due to starvation during winter: 77% for first-year colonies and 90% for established colonies (Seeley 1978, 1983). There is a high energetic cost of maintaining proper brood nest or cluster temperature during the cold winter. In addition, the high energetic cost of winter survival in temperate regions may greatly reduce the survivorship of small, secondary swarms or afterswarms, which would greatly reduce the net reproductive rate of honey bees in temperate regions. In contrast, periods of resource dearth in the tropics are not only shorter, but require less stored honey (per unit time) to enable the colonies to survive because of reduced energetic costs for maintaining brood nest temperature.

The reproductive season in French Guiana, South America, was nine months (Winston 1980b) compared with two to four months for North America (references cited in Winston 1980b and Winston, Dropkin and Taylor 1981). Is it a coincidence that the difference in the annual reproductive rate of Africanized bees in South America compared with European bees in North America, 16 vs. 3.0-3.6, respectively (Otis 1982a; Winston 1980a; Winston, Taylor and Otis 1983), is approximately of the same order (factor of 4-5) as the difference in the length of the reproductive season between South America and many areas of North America?

Another factor affecting differences in reproductive rates between honey bee populations in South America with those in North America is

the extent to which brood rearing ceases during resource dearths. European honey bee populations in temperate areas have a distinct seasonal decline in brood production and may stop brood rearing altogether for a variable period during winter (Bodenheimer 1937; Bodenheimer and Ben-Nerya 1937; Jeffree 1955; McLellan 1978; Nolan 1925, 1928). On the other hand, Winston reports that many Africanized colonies in French Guiana

> persist during the relative dearth season (March to June) without the cessation of brood rearing characteristic for temperate conditions, and are strong enough (i.e., have a relatively high worker population and sufficient young workers) to grow rapidly to swarming strength when resources improve. (Winston 1980b, p. 164).

This difference between tropical and temperate conditions allows tropical honey bee colonies to grow rapidly when resources become available and thereby increase their potential reproductive rates compared with temperate honey bee colonies.

More recently, investigations of both Africanized and European honey bee populations under identical experimental conditions in Venezuela have been undertaken. These studies have evaluated both demographic parameters as well as resource utilization behaviors. These investigations include the research presented in Chapters II-VII; studies by Winston and Katz (1981, 1982); and the research by the U.S. Department of Agriculture Bee Breeding and Stock Center Laboratory (Collins, Rinderer, Harbo and Bolten 1982; Harbo, Bolten, Rinderer and Collins 1981; Rinderer, Bolten, Collins and Harbo 1984; Rinderer, Bolten, Harbo and Collins 1982; Rinderer, Collins, Bolten and Harbo 1981; Rinderer, Collins and Tucker 1985; Rinderer, Tucker and Collins 1982). Results of these investigations present quite a different

picture as to the factors leading to the success and resulting impact of the Africanized honey bee in South America.

Factors Contributing to the Selective Advantage of Africanized Honey Bees in South America

Colony Demography

Table 8-1 summarizes the factors affecting colony survival and reproductive success for both Africanized and European honey bees under tropical conditions in Venezuela. Studies comparing parameters of colony demography for Africanized and European honey bees under identical conditions in Venezuela have produced surprising results (see Chapters II-VI). These studies were based on the assumption that the life history of the Africanized honey bee population in South America (as well as the parental population in Africa) was characterized by a high reproductive rate. Demographic features that were expected to be correlated with this high rate of colony reproduction, or short swarm to swarm interval, were shorter worker bee development time, smaller worker bee size, more rapid queen development and maturation, increased egg laying and brood production, reduced brood mortality, and increased adult worker bee longevity.

Colony demographic characteristics can be divided into two groups: those affecting the rate of colony growth and those affecting the time interval from swarming to the beginning of adult population increase. A rapid colony growth rate is most important for a high colony reproductive rate and is primarily a function of queen fecundity, adult worker longevity, and brood mortality (Brian 1965; Moeller 1961; Wilson 1971).

Although Africanized queens have been reported to have greater egg laying rates than European queens (Fletcher 1978; Michener 1972, 1975; Ribbands 1953), under identical experimental conditions in Venezuela, there was no significant difference in queen fecundity during the initial colony growth period (Chapters V and VI). Also, European honey bee workers live longer (Winston and Katz 1981), giving European honey bee colonies a growth rate advantage with respect to this demographic parameter.

Because of the relationship of worker longevity to colony growth rates, initial colony growth rates may be affected by the age structure of bees in a swarm. Colonies established from swarms with older bees will have a more rapid decline in population, which will adversely affect colony growth because egg laying rates are a function of the number of bees in a colony (Moeller 1958). The age structure of Africanized swarms has been evaluated (Winston and Otis 1978) but there are no data for both Africanized and European swarms under similar conditions.

Another parameter affecting colony growth rate is brood mortality, but there are no data available that simultaneously compare Africanized and European honey bees under identical conditions. Experimental and environmental conditions are particularly important with respect to this parameter. Rates of brood mortality can be as high as 50% and are affected by season, resource availability and colony adult population (Garofalo 1977; Merrill 1924; Woyke 1977). These high rates of brood mortality and/or brood cannibalism may function to regulate protein balance in honey bee colonies during protein (pollen) shortages (Weiss 1984). Therefore, earlier studies comparing brood mortalities in

Africanized and European colonies (Winston, Dropkin and Taylor 1981), which were observed under very different conditions, need to be re-evaluated, and new studies should be undertaken.

Also affecting colony growth rates is the extent to which brood rearing ceases during periods of resource dearth. As discussed earlier, Winston (1980b) reports that Africanized bees do not cease brood rearing to the extent observed for European bees and are therefore capable of rapid colony growth when conditions improve. European honey bees, under some tropical conditions, may have a sharp decline in brood rearing during resource shortages (Otis and Taylor 1979). However, these differences in brood rearing were not apparent when both Africanized and European honey bees were managed under identical conditions in Venezuela (Bolten, personal observation). Therefore, this behavior needs to be analyzed with both honey bee populations under a variety of tropical resource conditions to determine if there are differences, and whether the differences are a function of foraging behavior (see below) and/or intrinsic demographic parameters.

The two most important factors affecting the interval from swarming to the beginning of adult population increase are worker development time and queen maturation (Chapters II and IV). Worker development time has previously been considered an important factor affecting the rate of colony growth (Fletcher 1977a, 1978; Fletcher and Tribe 1977a; Tribe and Fletcher 1977; Winston 1979b; Winston, Dropkin and Taylor 1981; Winston and Katz 1982; Winston, Taylor and Otis 1983). As discussed in Chapter II, this is incorrect and is probably a result of confusing models for colony growth (increase in the number of bees in the colony) with models for population growth (increase in the number of colonies). Population

growth models are designed for other species in which all individuals are potential reproductives. For honey bees, individual (or worker bee) development time is not equal to generation time. Worker bee development time only affects the interval between a given change in egg laying rate and its resulting change in rate of adult emergence. The difference in worker development time between Africanized and European honey bees is only one day (Chapter II) and is trivial with respect to other factors affecting reproductive rates.

Time from virgin queen emergence to initiation of oviposition also only affects the interval from swarming until adult population increase begins and not the rate of colony growth (Chapter IV). The results presented in Chapter IV for maturation rates of Africanized and European queens were unexpected. European honey bee queens began oviposition at an earlier age post-emergence than did Africanized queens.

Reproductive Output

Reproductive output is mainly determined by the swarm to swarm interval and the number of swarms produced per swarming cycle. The swarm to swarm interval is a function of all the colony demographic parameters discussed in the previous section and resource utilization parameters discussed in the next section. Although there are no data on the swarm to swarm intervals for Africanized and European honey bees under identical conditions, results from investigations of demographic parameters reported above suggest that if differences in swarm to swarm intervals exist, they would not be a result of demographic differences. Rather, if differences exist, they are hypothesized to be a result of differences in resource utilization between the two populations (see below).

Although Africanized and European honey bees have not been compared under identical conditions, European colonies in North America (Kansas) produced the same number of small, secondary swarms, or afterswarms, per swarming cycle as did Africanized colonies in South America (French Guiana) (Otis 1980; Winston 1980a; Winston, Dropkin and Taylor 1981). These results are not directly comparable, but they do demonstrate that, at least under certain conditions, European honey bees can produce as many afterswarms as Africanized honey bees. Whether the number of afterswarms for European bees would be similar to Africanized bees under identical conditions needs to be analyzed. As discussed above, survivorship of small afterswarms would be much lower in temperate regions than in tropical regions, because of the energetic demands of temperate winters on honey bee colonies.

Resource Utilization

The most important factors leading to the success of Africanized honey bees in South America are associated with resource utilization: foraging behavior, brood production efficiency, worker bee size and absconding behavior. Although evaluated under different conditions, the foraging range of the parental African population is similar to that of European bees (Smith 1958b). However, under resource conditions typical of tropical regions, the foraging behavior of Africanized honey bees is significantly more successful than that of European honey bees (Rinderer, Bolten, Collins and Harbo 1984; Rinderer, Collins and Tucker 1985). Their success is a result of more frequent solitary foraging and reduced recruitment when resources are dispersed and limited, as is characteristic of most tropical habitats (Rinderer, Bolten, Collins and Harbo 1984; Rinderer, Collins and Tucker 1985). Using artificial

flowers, Nunez (1973, 1979a, 1982) analyzed foraging behaviors of Africanized and European honey bees. Differences between the two populations were observed that were appropriate to having evolved under either temperate or tropical resource availability patterns. Hoarding cage studies also demonstrated differences in response between Africanized and European honey bees, suggesting differences in resource utilization behavior (Rinderer, Bolten, Harbo and Collins 1982).

Increased numbers of bees in a colony are important for successful foraging, colony defense and reproduction (see Wilson 1971). When floral resources (nectar and pollen) are limited, a greater number of individual bees can be produced from a given amount of food if brood production is more efficient and/or if bees are smaller. There are no data available that were collected under identical conditions that allow comparison of brood production efficiency between Africanized and European honey bees, as measured by the ratio of developing brood to adult population for a range of different adult populations (Michener 1964; Moeller 1961). However, their smaller size may result in increased brood production efficiency in Africanized bees, i.e., less food is necessary to produce smaller bees (Chapter III; Fletcher and Tribe 1977a; Tribe and Fletcher 1977). Therefore, with a limited food supply, Africanized honey bees could increase their population at a greater rate than European honey bees. With more successful foraging behavior and smaller bee size, Africanized colonies can grow rapidly under conditions where European colonies may not be able to survive.

Another important difference between Africanized and European honey bees is the strategy used to survive during periods of food shortage. Honey bee colonies may either hoard sufficient quantities of food

(primarily honey) to sustain them during periods of resource dearth or the colonies can abscond (relocate or migrate) to another area where conditions may be better. Hoarding large surpluses of honey is characteristic of European honey bees in temperate regions. However, hoarding behavior may be disadvantageous in the tropics because colonies with large food surpluses may be more easily discovered by predators and less easily protected. Because predation has been a major evolutionary force for tropical honey bee populations (Seeley 1983; Seeley, Seeley and Akratanakul 1982), resource-induced absconding may be a better evolutionary alternative to hoarding.

Resource-induced absconding occurs when an entire colony abandons a nest and is quite common in tropical species of Apis (A. florea, A. dorsata, and A. cerana) and tropical populations of A. mellifera during periods of resource dearth (Winston, Otis and Taylor 1979; Winston, Taylor and Otis 1983; Woyke 1976). Africanized honey bees have a high rate of resource-induced absconding in South America (Winston, Otis and Taylor 1979). Resource-induced absconding behavior may not be advantageous in temperate regions because colonies may not have enough time once they settle in a new area to store sufficient food to successfully overwinter (Butler 1974). European honey bees generally do not abscond in either temperate or tropical regions (Butler 1974; Fletcher 1978; Winston, Otis and Taylor 1979; Winston, Taylor and Otis 1983).

Whether resource-induced absconding behavior is a more successful strategy in the tropics than is hoarding behavior requires further study to determine the advantages and disadvantages of each strategy under tropical conditions. Both may be viable strategies and may not be

mutually exclusive (Winston, Otis and Taylor 1979). Fletcher suggests that resource-induced absconding may not always be appropriate, considering

> the distance that bees can fly in relation to the general distribution of their food plants. The maximum flight range is unlikely to exceed about 16 kilometres...and yet huge areas of Africa inhabited by honey-bees consist of more or less uniform grasslands and savannah. With certain exceptions, therefore, such as movements up and down mountain slopes and in and out of river valleys, there would appear to be little advantage in absconding in such areas, for within their flight range the bees would very often find only more of the same type of country they had left. (Fletcher 1975, p. 13).

However, based on measurements of engorgement and estimates of metabolic rates, the maximum flight range of absconding colonies of honey bees has been calculated to be as great as 131 km (Otis, Winston and Taylor 1981). In addition, periodic foraging while in-transit could extend the potential distance even further. Until comparative studies demonstrate the advantages of either resource-induced absconding or hoarding, resource-induced absconding behavior, which is common in tropical honey bee populations, is concluded to be an advantageous behavior under some tropical conditions.

Predation and Colony Defense

Ability to defend the nest from predators affects colony survival and therefore reproduction. The colony defense behavior characteristic of Africanized honey bees (Collins, Rinderer, Harbo and Bolten 1982; Stort 1974, 1975a, 1975b, 1975c, 1976) may be more effective than that of European honey bees. One aspect of colony defense behavior of Africanized honey bees, their stinging behavior, is so extreme that Africanized bees are a public health hazard for both humans and domestic animals (Taylor 1986). This colony defense behavior is particularly effective against vertebrate predators. Africanized honey bees in

Venezuela also reduce the size of their nest entrances to a greater extent than European honey bees, which helps to protect against invertebrate predators, particularly ants (Bolten, personal observation).

Besides colony defense, another response to predation is to abscond (relocate). Because predation by both vertebrates and invertebrates and infestation by wax moths (Galleria mellonella and Achroia grisella) on honey bee colonies is extensive in tropical regions, disturbance-induced absconding would be an advantageous behavior and is frequently observed in tropical honey bees (Fletcher 1976; Seeley 1983; Seeley, Seeley and Akratanakul 1982; Winston, Taylor and Otis 1983). Disturbance-induced absconding was more frequently observed in Africanized honey bees than in European honey bees under similar conditions in Venezuela, particularly with respect to attacks by ants (Bolten, personal observation).

In addition to predation on the colony-level, Africanized worker bees may have behaviors that are better adapted to avoiding predators and parasites while foraging. The rapid, zig-zag flight of worker bees in the African parental population may be more advantageous in avoiding predators (invertebrate as well as vertebrate) compared with the slower, less erratic flight of European honey bees (Fletcher 1977b). Also, queen honey bees on mating flights are susceptible to predators. Fletcher (1977b) suggests that queens from the African parental population have shorter mating flights than European queens which may reduce predation. The differences in flight patterns and behaviors of Africanized and European honey bees need to be investigated under similar conditions.

Nest Sites and Cavity Volume

Fletcher (1976) suggested another adaptive advantage that Africanized bees have is their ability to utilize a greater variety of nest sites. Fletcher may be confusing cause with effect when he suggests that it is this ability that "has enabled them to establish themselves in areas not previously inhabited by honey-bees at all" (1976, p. 6). A more likely explanation for the success of Africanized bees in those areas would be their ability to utilize the particular nectar and pollen resources available. That is, without a more efficient utilization of resources, Africanized honey bees would not be able to exploit these other habitats irrespective of their ability to utilize a greater variety of nest sites. As discussed above, Africanized honey bees are more successful foragers than are European honey bees under resource conditions typical of tropical habitats.

Nest cavity volume is another factor affecting reproduction in honey bees. One of the stimuli for reproductive swarming is brood-nest crowding (Baird and Seeley 1983; Simpson 1966, 1973; Simpson and Riedel 1963; Winston and Taylor 1980). Colonies inhabiting smaller cavities become crowded more rapidly and have a higher tendency to swarm. Colonies established in large cavities would be less crowded and have a lower rate of swarming. In temperate regions, small cavities would be selected against because there would be less volume available for storing surplus honey to enable the colony to overwinter. Therefore, Seeley proposed that nest-cavity volume may "regulate mature colony size at an optimum between small colonies with low survivorship and large colonies with low fertility" (Seeley 1977, p. 226). Jaycox and Parise (1980, 1981) found that honey bees from northern Europe selected larger

nest cavities than did honey bees from southern Europe. Southern European winters would be much less severe than those in northern Europe, and therefore the need for larger nest cavities to store large food surpluses is less important.

For tropical honey bee populations, there may be a selective advantage for smaller nest cavity volumes, e.g., to facilitate protection against infestation from wax moths (Galleria mellonella and Achroia grisella) (Fletcher 1976). Africanized honey bees utilize a wider variety of nest sites than do European honey bees, including smaller cavity volumes (Fletcher 1976). The negative factors associated with smaller nest cavities may be absent in the tropics because there is less need to store large surpluses to survive periods of resource dearth--periods are generally shorter and less costly with respect to energetic demands for maintaining proper brood nest temperature. In addition, honey bees that evolved in the tropics commonly abscond during periods of resource dearth as opposed to hoarding surplus food.

Although nest cavity choice for Africanized and European honey bees has not been studied under identical conditions, nest cavities selected by Africanized honey bees in Venezuela were not smaller than cavities selected by European honey bees in temperate regions (Rinderer, Collins, Bolten and Harbo 1981; Rinderer, Tucker and Collins 1982). Because of the importance of nest cavity volume to reproductive rates, nest cavity volume for both populations needs to be investigated under identical conditions.

Density-Dependent Factors Regulating Queen Rearing

Other parameters that might account for differences in reproductive rates between Africanized and European honey bees may be certain

density-dependent factors that are responsible for regulating queen rearing in colonies preparing to reproduce. In European honey bees, initiation of queen rearing prior to reproduction is not a result of a decrease in queen pheromone production (Seeley and Fell 1981). Two other possibilities are suggested by Seeley and Fell (1981). First, there may be failure to adequately disperse queen pheromone in crowded colonies prior to swarming. And, second, worker bee response to queen pheromone may change prior to swarming.

Threshold levels for queen pheromone that inhibit queen rearing in worker bees may be different for Africanized and European honey bees. Also, dispersal of queen pheromone by "messenger" bees (Seeley 1979) may be different for Africanized bees compared with European bees. Baird and Seeley (1983) developed an equilibrium model to explain the regulation of queen rearing in colonies preparing to reproduce. Their model postulated that "there is a balance between nurse bees becoming inhibited from queen rearing and nurses losing their inhibition, and that whether a colony does or does not rear queens reflects the equilibrium percentage of inhibited nurses" (Baird and Seeley 1983, p. 221). Therefore, differences between Africanized and European honey bees with respect to density-dependent factors regulating queen rearing may result in differences in reproductive rates by affecting: 1) adult population size when colonies reproduce; 2) prime swarm size; and 3) number of afterswarms. Some of these density-dependent factors have been compared for Africanized bees in South America with European bees in North America under different environmental and experimental conditions (Winston, Dropkin and Taylor 1981). Unfortunately, there are no data collected under identical conditions that allow valid

comparisons to be made between Africanized and European honey bees that enable any density-dependent factors responsible for the reproductive rates and success of Africanized honey bees in South America to be identified.

Conclusion

In tropical regions, the success of Africanized honey bees compared with European honey bees is not a function of any intrinsic differences in colony demography. Rather, it must be concluded that the success of Africanized honey bees is due primarily to their ability to efficiently utilize tropical resources, enabling them to survive and reproduce under conditions where European honey bees are frequently not able to survive. If European honey bee colonies are not able to survive and/or grow under some of the tropical resource conditions of South America, they obviously cannot reproduce. It is precisely because the European honey bees were not successful foragers (= honey producers) in most tropical regions of Brazil that African honey bees were imported into Brazil (Goncalves 1974, 1975, 1982; Woyke 1969).

The efficient utilization of tropical resources by Africanized honey bees is a result of a set of adaptive behaviors involving solitary foraging, reduced recruitment, increased brood production efficiency because of smaller worker bee size, and both resource-induced and disturbance-induced absconding. These characteristics, combined with an effective colony defense behavior, give Africanized honey bees a selective advantage that results in increased survivorship, increased colony growth rates and ultimately increased reproduction, which is responsible for their rapid dispersal and high population densities.

The Africanized honey bees studied in Venezuela are only a small sample of the total Africanized honey bee population in South and Central America and represent only a fraction of the variation within the population, particularly if we consider that Africanized honey bees are a result of hybridization. Nevertheless, the results presented in the foregoing chapters demonstrate that at least some portion of the Africanized honey bee population is similar to the European honey bee population with respect to the demographic parameters analyzed.

Potential Impact of Africanized Honey Bees in North America

The selective advantage of Africanized honey bees in South America will be lost as they disperse north into temperate regions. European honey bees will have the selective advantage in temperate regions because of their particular behavioral repertoire which is better adapted to temperate conditions. However, because the populations can interbreed successfully, negative characteristics of the Africanized population, e.g., their stinging behavior, may become genetically introgressed into the European population of North America and therefore widespread wherever honey bees can survive (Chapter VII). A more optimistic scenario is that the large population of European honey bees in Mexico will slow the spread of African genes because of competition for available resources as well as through hybridization. Therefore, through selection, hybridization, and competition, the impact of Africanized honey bees may be minimized in North America (Chapter VII).

TABLE 8-1. Factors affecting colony survival and reproductive success for Africanized and European honey bees in Venezuela.

FACTOR	POPULATION WITH ADVANTAGE	REFERENCES
COLONY DEMOGRAPHY		
Growth Rate		
Egg Laying Rate	No Difference	Chapter VI; Chapter V
Worker Longevity	European	Winston & Katz 1981
Swarm Age Structure	No Data	
Brood Mortality	No Data	
Brood Production during Resource Dearth	No Data	
Interval[a]		
Worker Development Time	Africanized	Chapter II
Queen Maturation	European	Chapter IV
REPRODUCTIVE OUTPUT		
Number of Afterswarms per Swarming Cycle	No Data	
RESOURCE UTILIZATION		
Foraging Behavior	Africanized	Rinderer, Bolten, Collins & Harbo 1984; Rinderer, Bolten, Harbo & Collins 1982; Rinderer, Collins & Tucker 1985; Nunez 1979, 1982; Winston & Katz 1982
Brood Production Efficiency	No Data	
Bee Size	Africanized	Chapter III
Resource-Induced Absconding	Africanized	Winston, Otis & Taylor 1979 Winston, Taylor & Otis 1983
PREDATION		
Colony Defense	Africanized	Collins, Rinderer, Harbo & Bolten 1982
Disturbance-Induced Absconding	Africanized	Bolten, pers. observation; Winston, Taylor & Otis 1983
Flight Behavior	No Data	
NEST CAVITY VOLUME	No Data	
DENSITY-DEPENDENT FACTORS	No Data	

[a]Interval from swarming to beginning of population increase.

APPENDIX A
WORKER BEE DEVELOPMENT TIMES AND MORTALITY DURING DEVELOPMENT

TABLE A-1. Comparison of worker bee development time (in days) for Africanized and European honey bees: median, (range), mean ± SD, (n = sample size). All development measured in European comb cell size with European nurse bees.

EGG GENOTYPES	UNSEALED BROOD	SEALED BROOD	TOTAL DEVELOPMENT[a]
AFRICANIZED			
A53 (n = 25)	5.0 (4-5) 4.8 ± 0.4	11.0 (11-12) 11.4 ± 0.5	19.0 (19-20) 19.2 ± 0.4
A26 (n = 9)	5.0 (5) 5.0 ± 0	11.0 (11-12) 11.3 ± 0.5	19.0 (19-20) 19.3 ± 0.5
A25 (n = 19)	5.0 (4-5) 4.6 ± 0.5	11.0 (11-12) 11.4 ± 0.5	19.0 (19) 19.0 ± 0
COMBINED (n = 53)	5.0 (4-5) 4.8 ± 0.4	11.0 (11-12) 11.4 ± 0.5	19.0 (19-20) 19.2 ± 0.4
EUROPEAN			
W18 (n = 28)	5.0 (4-5) 4.8 ± 0.4	12.0 (11-13) 12.0 ± 0.3	20.0 (19-20) 19.8 ± 0.4
H1 (n = 19)	5.0 (5-6) 5.4 ± 0.5	12.0 (11-13) 12.1 ± 0.4	20.0 (20-21) 20.5 ± 0.5
Y(A5) (n = 26)	5.0 (4-5) 4.8 ± 0.4	12.0 (11-12) 12.0 ± 0.2	20.0 (19-20) 19.8 ± 0.4
COMBINED (n = 73)	5.0 (4-6) 5.0 ± 0.5	12.0 (11-13) 12.0 ± 0.3	20.0 (19-21) 20.0 ± 0.5
ANALYSES[b]	NS	P<0.001	P<0.001

[a]Total development = time from oviposition to adult emergence.
[b]Kolmogorov-Smirnov one-tailed test, chi-square distribution, df = 2, alpha = 0.05 (Siegel 1956). Combined samples used for analyses.

TABLE A-2. Mortality during different developmental stages. Mortality was measured in European comb cell size with European nurse bees.

	E_1[a]	E_2[b]	L_1[c]	L_2[d]	SB[e]	N[f]
AFRICANIZED EGG GENOTYPES						
A53	0	0	5	0	0	30
A26	0	0	--	19[g]	0	28
A25	1	0	9	0	0	29
EUROPEAN EGG GENOTYPES						
W18	0	0	0	2	0	30
H1	0	0	3	8	0	30
Y(A5)	0	0	4	0	0	30

[a]Mortality during first 24 hours in test colony (acceptance).
[b]Mortality between 24-72 hours (before hatching).
[c]Mortality between 72-96 hours (at time of hatching).
[d]Mortality during older larval stages, before sealing.
[e]Mortality during the pupal stage.
[f]N = total eggs monitored.
[g]Not distinguished between L_1 and L_2.

APPENDIX B
HONEY BEE SIZE, COMB CELL SIZE AND SIZE VARIATION

TABLE B-1. Coefficients of variation (CV) of worker bees in honey bee and bumble bee colonies calculated from data presented in the references.

	CV	REFERENCES
Honey bees (Apis mellifera)		
Weights		
Adult (fresh)	0.4-0.6	Abdellatif 1965
	4.0-4.5[a]	Bolten (Table B-2)
	5.4-7.0[b]	Bolten (Table B-2)
	10.7-11.2[c]	Kerr and Hebling 1964
Adult (dry)	4.8-8.1	Grout 1937
	4.1-4.5[a]	Bolten (Table B-2)
	4.1-6.7[b]	Bolten (Table B-2)
Linear Measurements		
Length forewing	1.5-1.6	Grout 1937
Width forewing	2.2-2.5	Grout 1937
Length proboscis	1.6-1.9	Grout 1937
Bumble bees (Bombus)		
Weights		
Adult (fresh) (Bombus agrorum)	31.0-36.7	Brian 1952
Linear Measurements[d]		
Length radial cell (Bombus fervidus)	7.4-13.8	Medler 1965

[a]European genotypes.
[b]Africanized genotypes in South America.
[c]CV may be high as a result of variable engorgement during 4 hour delay from emergence to weighing.
[d]Different linear measurements for Bombus are significantly correlated ($P<0.01$, Medler 1962).

TABLE B-2. Africanized and European adult honey bee weights (mg): mean ± SD, coefficient of variation (CV), (sample size).

GENOTYPE	COMB CELL TYPE[a]	FRESHLY EMERGED	DRIED[b]	CORRELATIONS[c]
AFRICANIZED				
A26	EUR	94.9 ± 5.1 CV = 5.4 (28)	13.5 ± 0.6 CV = 4.4 (28)	***
A57	EUR	88.6 ± 6.2 CV = 7.0 (30)	11.9 ± 0.8 CV = 6.7 (30)	***
B39	EUR	87.4 ± 4.7 CV = 5.4 (16)	12.3 ± 0.5 CV = 4.1 (16)	NS
A60	AFR	95.2 ± 5.1 CV = 5.4 (30)	13.2 ± 0.6 CV = 4.5 (30)	***
EUROPEAN				
WE2	EUR	107.1 ± 4.8 CV = 4.5 (29)	14.6 ± 0.6 CV = 4.1 (29)	**
Y(K)	EUR	116.1 ± 4.7 CV = 4.0 (27)	15.5 ± 0.7 CV = 4.5 (27)	***

[a]EUR = European comb cell diameter = 5.4 mm.
AFR = Africanized comb cell diameter = 4.8 mm.
[b]Dried at 50°C for 48 hrs.
[c]Pearson's correlation coefficient, alpha = 0.05;
** = $P<0.01$; *** = $P<0.001$.

TABLE B-3. Comparison of Africanized and European comb cell diameter and comb cell volume: mean ± SD, range, CV, (sample size).

	DIAMETER[a] (mm)	VOLUME[b] (ml × 10^{-3})	CORRELATIONS[c]
AFRICANIZED COMB CELLS	4.8 ± 0.1 4.6 - 4.9 CV = 2.1 (50)	184.6 ± 15.8 160 - 215 CV = 8.6 (50)	NS
EUROPEAN COMB CELLS	5.4 ± 0.05 5.4 - 5.5 CV = 1.0 (30)	264.3 ± 23.5 225 - 300 CV = 8.9 (30)	NS[d]
ANALYSES[e]	P<0.001	P<0.001	

[a]Determined by measuring 10 horizontal, consecutive cells; cell-wall thickness not considered.
[b]Cell volume determined by filling cells with water with a pipette.
[c]Spearman's rank correlation coefficient, alpha = 0.05.
[d]Negative correlation, P<0.01.
[e]t-test, one-tailed.

TABLE B-4. Comparison of Africanized and European comb cell diameter and comb cell depth: mean ± SD, range, (sample size).

	DIAMETER[a] (mm)	DEPTH (mm)	CORRELATIONS[b]
AFRICANIZED COMB CELLS	4.8 ± 0.1 4.7 - 4.9 (17)	11.8 ± 0.2 11.4 - 12.3 (17)	NS
EUROPEAN COMB CELLS	5.4 ± 0.05 5.3 - 5.4 (26)	12.2 ± 0.4 11.5 - 12.8 (26)	NS
ANALYSES[c]	P<0.001	P<0.001	

[a]Determined by measuring 10 horizontal, consecutive cells; cell-wall thickness not considered.
[b]Spearman's rank correlation coefficient, alpha = 0.05.
[c]t-test, one-tailed.

TABLE B-5. Changes in European worker bee pupal weight (mg) with changes in pupal age: mean ± SD, (sample size). Fresh weights were measured in Gainesville, Florida.

AGE (DAYS POST-OVIPOSITION)						
11.5	12.5	13.5	14.5	15.5	16.5	17.5
145.8 ± 4.8 (10)	145.5 ± 3.8 (45)	141.9 ± 3.8 (31)	140.9 ± 4.2 (38)	141.1 ± 4.6 (31)	139.1 ± 4.0 (32)	138.0 ± 4.8 (34)
A	B	C	D	E	F	G

ANALYSES	CDEF	NS[a]
	A × B	NS[b]
	B × C	NS
	C × D	NS
	D × E	NS
	E × F	NS
	F × G	NS

[a]One-way analysis of variance, alpha = 0.05.
[b]t-test, two-tailed, alpha = 0.05.

APPENDIX C
CHANGES IN QUEEN PUPAL WEIGHT WITH AGE

TABLE C-1. Changes in European queen pupal weights (mg) with changes in age: mean ± SD, (sample size). Queen pupal weights were measured in Baton Rouge, Louisiana.

DAYS POST-OVIPOSITION					
9	10	11	12	13	14
311.6 ± 12.4 (10)	291.6 ± 8.3 (10)	294.6 ± 9.7 (10)	293.4 ± 8.4 (10)	287.4 ± 11.2 (10)	274.1 ± 16.9 (5)
A	B	C	D	E	F
ANALYSES[a]		ABCDEF BCDE	P<0.001 NS		

[a]One-way analysis of variance, alpha = 0.05.

APPENDIX D

ACCURACY OF TECHNIQUE USED TO ESTIMATE NUMBERS OF BEES IN A COLONY

TABLE D-1. Accuracy of technique used to estimate number of bees in colony.

SAMPLE NO.	COUNTED	ESTIMATED[a]	DIFFERENCE	% DIFFERENCE
1	2284	2324	40	1.8
2	2906	2918	12	0.4
3	3085	3080	5	0.2
4	3079	3124	45	1.5
5	2657	2717	60	2.2
6	2046	2102	56	2.7

[a]The number of adult bees was estimated by determining the mean individual bee weight from three, 150-200 bee samples. The total weight of bees in each sample was then divided by the mean individual bee weight to get an estimate of the total number of bees in each sample.

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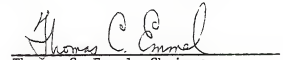

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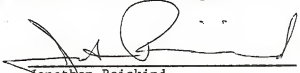

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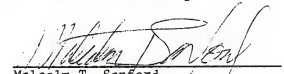

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This dissertation was submitted to the Graduate Faculty of the Department of Zoology in the College of Liberal Arts and Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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